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Artificial insemination of cattle

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ARTIFICIAL INSEMINATION OF CATTLE

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Introduction

Artificial insemination is generally considered to be the greatest single tool available to the dairy farmer for making mass improvement in the heredity of his cattle. Few, if any, practices designed to increase milk production and improve the type of cattle have enjoyed the popularity and general acceptance on the part of dairymen as has artificial insemination. The annual average milk production is 6,438 pounds for all dairy cattle in the United States, and is only 3,892 for the South Central States, including Louisiana. Milk production levels for commercial dairy cattle are somewhat higher than those given above. In comparison, the production level for all artificially sired daughters which have been tested is 10,583 pounds of milk per year. Bulls of this caliber will do much to increase efficiency of production.

Advantages of Artificial Insemination

There is general agreement among the leaders in the livestock industry that artificial insemination has several distinct advantages over natural mating:

1. The usefulness of outstanding sires is increased manifold. A sire possessing the ability to transmit high production and desirable type can be used to breed several thousand cows in many different herds each year. In natural mating, his service would be limited to 50 or 60 cows in one or two herds.

2. Commercial dairymen and small breeders have the services of much better bulls than they could afford to buy. Many dairymen are still using grade bulls or inferior registered sires on which little or no information is available concerning the production or type of their ancestors and close relatives.

3. The danger of spreading genital disease, such as vibriosis, is greatly reduced. All bulls used in organized artificial breeding associations are tested for all known diseases before being placed in service and at regular intervals thereafter. As an added measure of protection, all semen used in the Louisiana program is treated with antibiotics that will destroy any disease producing bacteria that might be present.

ACKNOWLEDGMENT—The authors wish to acknowledge and express their appreciation to Lorraine Boss Allen for making the drawings and to Dr. J. E. Johnston for doing the photographic work for this publication. Appreciation is also expressed to Drs. J. B. Frye, Jr., and J. E. Johnston for their suggestions and criticisms of the manuscript.

4. Artificial insemination eliminates the trouble, danger, and expense of keeping a bull. The dairy bull is the most troublesome and dangerous animal on the farm. Many farm people are killed or seriously injured by bulls each year. In most cases, artificial insemination actually costs less than natural mating. The average cost of keeping a bull on the farm is $190 per year. This includes feed, pasture, and labor but does not include the purchase price of the bull. A dairyman in Louisiana can breed a herd of 30 cows artificially for $150 per year or less. This would result in a saving of $40 per year over and above the original cost of the bull. The price of a well-bred young bull ranges between $400 and $2,000, depending upon the breed, pedigree, bloodline, and age. Well-proved sires range in price from $2,000 to $15,000.

5. With artificial insemination, the dairyman can breed his cows to several bulls. Breeding all of your cows to one bull is like "putting all of your eggs in one basket."

6. Yearling heifers or small cows may be bred to large bulls without difficulty or injury. In natural mating, many dairymen keep an extra young bull to breed to heifers. This is not necessary in artificial insemination.

7. The transmitting ability of a bull can be determined quickly and more effectively in artificial insemination. Proof based upon the production of artificially sired daughters located in many different herds is much more reliable than natural proof where the daughters are tested in one or two herds.

8. Selective mating of outstanding animals, located thousands of miles apart, is possible. Several Louisiana breeders have obtained fresh and frozen semen from outstanding bulls located throughout the United States for special matings.

9. In most cases, more complete breeding and calving records are kept when cows are bred artificially.

10. A dairyman can keep several different breeds without any added expense. In natural mating, he would need a bull for each breed of cows or would be forced to practice crossbreeding.

11. Dairymen take pride in their artificially sired heifers and, consequently, do a better job of feeding and managing them.

Limitations of Artificial Insemination

Although artificial insemination is a powerful tool for making mass improvement in the heredity of dairy cattle, certain disadvantages must be considered:

1. To be successful, all phases of artificial insemination must be carried out by a well-trained operator according to proven methods and practices.

2. Cows must be observed more closely for signs of heat and kept in or near the barn so as to be readily available for insemination.

3. In order to prevent infection and eliminate the spread of dis-ses, all equipment used must be clean, and good sanitary prac-es must be followed in each phase of the operation.

4. Artificial insemination reduces the demand for mediocre bulls t increases the demand for well-bred young bulls and desirably oved sires.

istorical Aspects

Artificial insemination is old in concept but relatively new in actice. Perry (20) reported that an Italian physiologist, L. Spal-nzani, successfully inseminated a female dog in 1780. However, ore than a century passed before this new method of breeding was ed as a means of improving livestock. E. I. Ivanoff, a Russian ientist, was a pioneer in artificial insemination. He was successful inseminating mares as early as 1899 and initiated a large-scale eeding project in cattle and sheep during the years prior to World ar I. Following the war, a new livestock breeding experiment sta-n was established in Russia with Ivanoff as its first director. By 38, the Russians were inseminating 120,000 mares, 1,200,000 cat-, and 15,000,000 sheep annually.

The first cooperative artificial breeding association in the world s organized in Denmark in 1936, with 1,070 cows being bred the st year. By 1960, Denmark had many cooperative breeding asso-tions and 99 per cent of the dairy cattle were being bred artifi-lly. Artificial insemination was begun in the United States on an anized basis in New Jersey in 1938, with 102 members and 1,050 ws enrolled. During the next few years, artificial insemination as-iations were organized throughout the United States. By January, 0, artificial insemination was being used in every state of the ion, with 64 organizations inseminating 6,932,294 cows in 946,000 ds annually. This means that one out of every three (or approxi-tely 33 per cent) of the dairy cows in the United States is being d artificially.

The exact number of beef cattle that are being bred artificially the United States is not available. However, almost all of the anized breeding associations in this country offer artificial serv-from two or three beef breeds. This program is handicapped by fact that the Angus and Hereford breed associations do not per-registration of artificially sired offspring unless both male and ale are owned by the same person. Many purebred beef breeders ctice artificial insemination in their own herds. It is permissible three or four breeders to own a bull jointly and use him to breed ebred cattle.

Research in artificial insemination was started by the Louisiana te University Dairy Department in 1943. The North Louisiana sey Breeder's Association, which was started in 1946 at Ruston,

Louisiana, was the first organized artificial insemination unit in this state. In 1946, the state Legislature appropriated funds and instructed the Dean of the LSU College of Agriculture to initiate a statewide livestock improvement program, including artificial insemination of cattle. A committee composed of livestock farmers and representatives of the extension and research divisions of the LSU College of Agriculture was appointed by the Dean and requested to make recommendations as to how this program could best be carried out. The committee recommended that a central bull stud be established on the LSU campus to service the entire state.

The Louisiana Artificial Breeding Cooperative, Inc., a companion organization to the Dairy Improvement Center, was organized July 16, 1947. This organization is a farmers' cooperative having as its main purpose the improvement of livestock through artificial insemination. Semen was first shipped from the LSU Dairy Improvement Center on October 15, 1947, to 15 local breeding circuits of the cooperative. Several other local circuits were organized shortly after the program was initiated, and a total of 12,653 cows were bred during the first year. Artificial insemination of cattle has expanded throughout the state; on January 1, 1960, there were 42 local breeding circuits employing 45 full-time technicians and with more than 51,000 cows being inseminated annually.

Louisiana has a unique artificial insemination program in that it is jointly administered by the Louisiana Artificial Breeding Cooperative, Inc., and the LSU College of Agriculture. The Dairy Department and the Agricultural Extension Service were designated to work with the Cooperative in carrying out this program.

The Dairy Improvement Center was established as a service unit of the LSU Dairy Department, with its main purpose being that of a bull stud supplying semen from outstanding bulls to organized groups throughout the state. Another function of the Center is that of conducting research. Much of the success which the artificial insemination program has achieved can be attributed to scientific investigations. Likewise, the future of this program is dependent upon the development of new and improved techniques. It is also the responsibility of the Center to receive, analyze, and file all artificial insemination records. Other responsibilities of the Center consist of assisting with the training and in the certification of breeding technicians and in teaching students enrolled in the College of Agriculture.

The Extension Service, through its dairy specialists and agricultural agents, is responsible for the organizational, educational, and promotional phases of the work. It is also responsible for assisting with the selecting of bulls, training technicians, and giving technical assistance and supervision to the breeding technicians in the field.

Structure and Functions of the Reproductive Organs

A general knowledge of the structure and functions of the male and female reproductive organs is essential in order to master the technique of artificial insemination. The reproductive processes in both the male and female are controlled by hormones produced by internal glands. These organs are called endocrine glands because their secretion is liberated directly into the blood stream.

Of the many known endocrine glands, the pituitary is considered to be the master gland since it influences or regulates many of the other glands. The pituitary gland is a small body enclosed in a bony structure at the base of the brain. It is divided into two lobes, the anterior and posterior. Secretions from the anterior lobe are best known for their effect on growth, sex glands, and milk secretion. As the animal reaches sexual maturity, the gonads, both testicles and ovaries, are activated by secretions from the pituitary gland. In the male, the gonadotropic hormone known as "F.S.H.," or follicle stimulating hormone, stimulates the male germ cells to produce spermatozoa. A second gonadotropic hormone, which is known as "I.C.S.H." or interstitial cell stimulating hormone, stimulates the testicles to produce the male hormone which is known as testosterone. Similarly in the female, the gonadotropic hormone "F.S.H." stimulates follicle growth in the ovary. As the follicle develops, the estrogenic hormone known as estradiol or estrogen is produced. The presence of estrogen in the blood stream stimulates the anterior pituitary to secrete a second hormone known as "L.H.," or luteinizing hormone. This hormone causes ovulation and stimulates growth of the granulosa cells to form the corpus luteum, or yellow body.

Male Reproductive Organs

The principal organs of reproduction in the male consist of the testicles, which are carried outside of the body in the scrotum. Spermatozoa are formed in a network of tubules located inside the testicles, and the male hormone, testosterone, is produced by the cells of Leydig (interstitial cells) which are found between the tubules. The development and behavior of the male are greatly affected by testosterone. Its chief functions are development of secondary sex characters, sex drive, and development and maintenance of the reproductive organs. Attached to each testicle is a coiled tube, the epididymis, which extends down the outside of the testicle to its base. Spermatozoa pass from the testicle to the epididymis, where they undergo a maturing process. Leading upward from the epididymis is a tube, the vas deferens, connecting with the urethra which passes through the penis and provides an opening to the exterior for ejaculation of semen and the passage of urine. The upper end of each vas deferens enlarges to form the ampulla, where spermatozoa are stored until ejaculation occurs. Located on either side of the

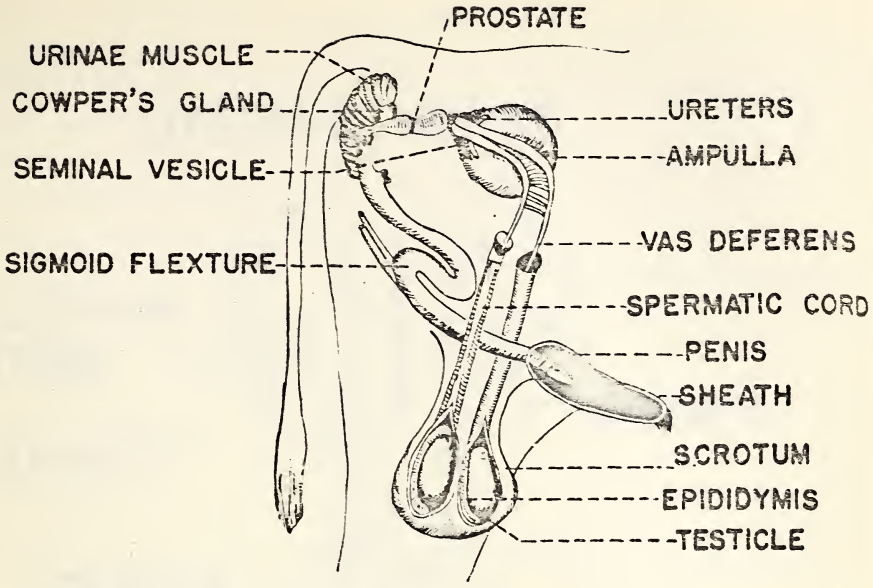

FIG. 1.— Reproductive organs of the bull.

ampullae are the seminal vesicles, which are the largest of the accessory glands. They produce a fluid containing fructose and citric acid which mixes with the spermatozoa and serves as a carrier. Other accessory glands include the prostate, which surrounds the urethra near the neck of the bladder, and the Cowper's glands located on either side of the urethra. These glands also secrete a fluid which serves as a carrier of the sperm. The prostatic secretion is alkaline in nature and is thought to be of value in neutralizing any acid condition that might be present in the urethra and vagina.

The penis of the mature bull is about one inch in diameter and three feet in length. Behind and slightly above the testicles, the penis forms an "S" shaped curve, the sigmoid flexure, which takes up about one foot of the organ when it is in the relaxed state. During natural mating or when semen is collected with the artificial vagina the curve is extended. Functions of the penis are to drain the bladder of urine and to introduce semen into the vagina of the cow. Semen is the normal discharge of the male during mating. It is a whitish, milk-like fluid which consists of the spermatozoa and the secretions from the seminal vesicles and the prostate and Cowper's glands. Spermatozoa are extremely small, being less than 1/500 of an inch in length, and shaped somewhat like a tadpole.

Female Reproductive Organs

The female reproductive tract is very complex; it not only produces the ovum (egg), but provides for the nourishment and growth

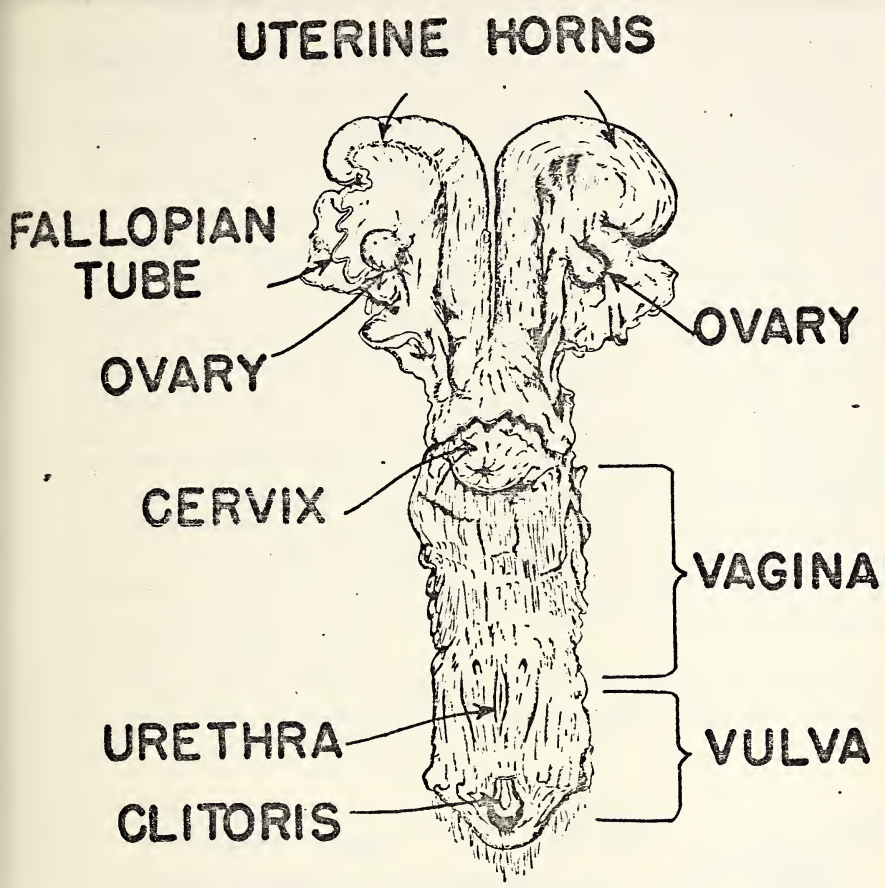

FIG. 2.—Reproductive organs of the cow.

of the fetus and at the end of pregnancy, expels the fully developed calf weighing up to 100 pounds or more. The essential organs of reproduction in a cow include the ovaries, oviducts, uterus, vagina, and vulva, as shown in Figure 2.

The two ovaries of the cow are located in the pelvic cavity suspended in the broad ligament. These organs vary considerably in shape and size but in general they are shaped like a slightly thickened lima bean, measuring 1½ inches x 1 inch x one-half inch. Germinal epithelium covers the surface of the ovary except at the point of attachment. This epithelium, together with the supporting connective tissue, makes up the outer layer (cortex) of the ovary. The inner layer (medulla) is composed of blood vessels, nerves, and connective tissue. The ovaries have a dual function. They not only provide the reproductive cells (ova or eggs) but produce hormones which prepare the tract to receive the fertilized egg, supply nourish-

ment, carry the fetus throughout pregnancy, and expel the fully developed calf.

Production of ova is a continuous process which starts before birth and extends throughout sexual life. An ovum develops from the germinal epithelium and soon becomes enclosed in a structure known as the ovarian follicle. At first the follicle consists of a single layer of cells surrounding the ovum. The follicle increases in size by multiplication of the epithelial cells and a special formation of the surrounding connective tissue. As growth continues, a cavity is formed which is filled with a light brown, alkaline fluid, the follicular fluid. Immature follicles range in size up to ½ inch in diameter. When the follicle reaches maturity, it is approximately ¾ inch in diameter and has the appearance of a blister. Normally only one follicle reaches maturity at a single heat period. It is generally believed that immature follicles degenerate, with new ones being formed for the next heat period. Ovulation takes place by the rupture of the follicle at the ovarian surface, and the ovum is washed out by follicular fluid into the upper end of the oviduct, where it is soon ready to be fertilized by the male sex cell. Immediately following ovulation, the corpus luteum (yellow body) forms from the walls of the follicle. It reaches maximum size (¾ inch to 1 inch in diameter) in 10 days and degenerates rapidly after 18 days unless conception takes place, in which case it remains fully developed throughout pregnancy.

The ovum is about 1/200 of an inch in diameter and can barely be seen with the naked eye. It is estimated that two million ova could be placed in a thimble.

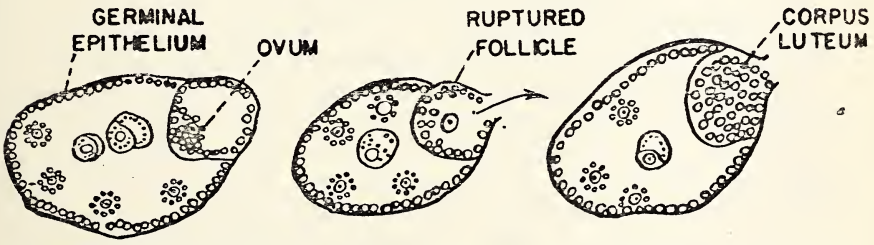

FIG. 3.—Sketch showing ovaries at various stages of the estrus cycle.

The oviducts are 8 to 10 inches long and about one-tenth of an inch in diameter. One end of the tube is a continuation of the uterine horn. The other end enlarges and fans out to form the fimbria, which serves as a funnel to catch the egg when it is released. These tubes contain no glands and practically no cillia. The spermatozoa and fertilized egg are transported by muscular contractions. Fertilization occurs in the upper end of the tube soon after

ovulation, with 4 to 6 days being required for the fertilized egg to reach the uterus.

The uterus consists of the body and two horns. The body is approximately 1½ inches long, but from the outside appearance, it looks to be 3 to 5 inches longer because of the close attachment of the horns. The horns are about 15 inches long and 1 to 1¾ inches in diameter. They taper and curve downwards and backwards, as shown in Figure 2.

The lining of the uterus has about 100 slightly raised areas which are known as "caruncles" or "cotyledons." During pregnancy, the cotyledons enlarge and take on a sponge-like texture to receive the villi of the fetal membrane.

The cervix serves as a barrier between the uterus and the vagina. This organ is composed of very thick muscular walls and is about 3 to 4 inches in length and 1 inch in diameter. The narrow canal has many longitudinal folds and 3 or 4 transverse folds, which makes the introduction of any instrument rather difficult. The cervix dilates sufficiently to allow passage of the fetus at calving time. There is also a slight relaxation of the cervix during estrus. This organ contains a large number of secreting cells and is the chief source of mucus during the heat period. During pregnancy, it is filled with a thick mucus plug which is referred to as the "cervical seal of pregnancy."

The vagina is 8 to 10 inches long and has many lengthwise folds. Transverse folds may be produced by abdominal straining. If these folds interfere with the passage of the inseminating tube, the cervix may be grasped and pushed forward. The posterior constrictor muscle which is found at the junction of the vagina and vulva serves to prevent the flow of urine forward into the vagina. The vagina receives the penis of the bull in natural mating and serves as a birth canal at parturition. The vulva is 4 to 5 inches long and serves as the external opening of the reproductive and urinary tracts. A small erectile organ, the clitoris, is located just inside the vulva. It is 4 to 5 inches long, but only the pointed end is visible in the vulva.

Hormones produced by the ovary play a very important role in regulating the reproductive processes. Estrogen is produced by the developing follicle of the ovary. Its chief functions are as follows: development of secondary sex characters, increase blood supply to the reproductive tract which stimulates growth of the uterine lining and increases muscle tone in the Fallopian tubes and uterine wall, aid in mammary development, and bring cow into heat. Progesterone is produced by the corpus luteum and has as its functions the preparation of the uterus to receive the fertilized egg, prevention of estrus and ovulation during pregnancy, and aid in the development of the mammary gland.

Reproductive Events

Puberty may be defined as the age at which the reproductive organs become functional. Most heifers experience their first estrus or heat period at 6 to 8 months of age. However, the attainment of sexual maturity is a gradual process, and puberty does not indicate full reproductive capacity.

A large majority of the non-pregnant cows come in heat every 18 to 24 days, with an average of 21 days. Unbred heifers have an average cycle length of 20 days, with 85 per cent falling between 18 and 22 days. The length of the heat period ranges from 2½ to 28 hours, with an average of 16 hours for heifers and 18 hours for cows. Work in progress at this station indicates that the duration of estrus in Louisiana may be somewhat shorter than that reported above. Symptoms of heat are restlessness, a tendency to mount or allowing other cows to mount, and a slight swelling and redness of the vulva, with a flow of clear mucus. Standing when mounted by another animal is the most accurate sign of estrus. Ovulation occurs 3 to 18 hours after the end of heat, the average being 10 to 12 hours for both cows and heifers.

The physiological processes of reproduction may be summarized as follows: (1) development of the follicle, (2) estrus or heat, (3) copulation or insemination, (4) ovulation, (5) fertilization, (6) formation of corpus luteum or yellow body, (7) implantation of fertilized egg, (8) gestation or pregnancy, (9) parturition, (10) lactation, and (11) absorption of the corpus luteum.

Collecting, Evaluating, Processing, and Shipping Bull Semen

Good quality semen is one of the primary requirements for success in artificial insemination. In order to obtain maximum breeding efficiency, it is necessary to use the correct techniques and sanitary precautions.

Collection of Semen

The three recognized methods of collecting semen from the bull are as follows: use of artificial vagina, the massaging of the ampullae, and electro-ejaculation (13). Use of the artificial vagina is the most satisfactory method of collecting semen from the bull and is universally practiced in artificial breeding associations and private herds. The artificial vagina consists of a heavy rubber cylinder 14 inches long and 2¾ inches in diameter with a thin rubber tubing folding over each end and secured with heavy rubber bands. A funnel-shaped piece of rubber tubing is slipped over one end of the vagina and held by a heavy rubber band. The sterile collecting tube, which is attached to the small end of the funnel, is usually covered with

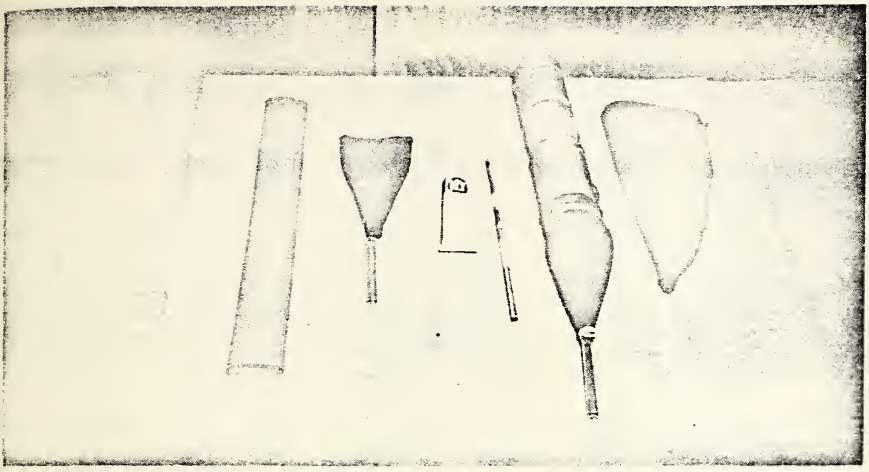

FIG. 4.—Equipment used in collecting semen. Left to right: rubber cylinder and bands, inner liner, funnel and graduated tube, lubricating jelly, glass rod for applying lubricant, artifical vagina assembled, and protector jacket.

flannel or paper towels to protect the semen against cold shock. During cold weather a protector jacket or a special type artificial vagina is used to prevent cold shock.

The desired temperature and pressure is usually obtained by filling the outer jacket about three-fourths full of water at a temperature of 120° F. For most bulls, the correct temperature of the inside of the vagina at the time of collection is 105° to 110° F. A thin coat of sterile lubricant such as KY jelly is spread over the upper one-half of the inner lining by means of a sterile glass rod just prior to collection. A quiet cow or a steer is commonly used for the bull to mount at the time of collection. Immediately before collection, the area around the sheath is washed with a solution containing 200 parts of chlorine per million to reduce bacterial contamination of the semen.

After proper stimulation, the bull is allowed to mount the cow, at which time the attendant places his hand on the sheath and directs the penis into the artificial vagina. As the bull makes his thrust, the semen is deposited in the funnel and collecting tube. Studies made at this station (6) showed that the average volume of semen per ejaculate was increased by 22 per cent and the average number of motile spermatozoa by 55 per cent when the bull was properly stimulated prior to the collection of the first ejaculate. The false mount—that is, allowing the bull to mount the dummy animal without serving—was found to be an effective method of obtaining the desired level of sexual excitement. In the false mount, the penis is guided to the side of the collection animal by the attendant.

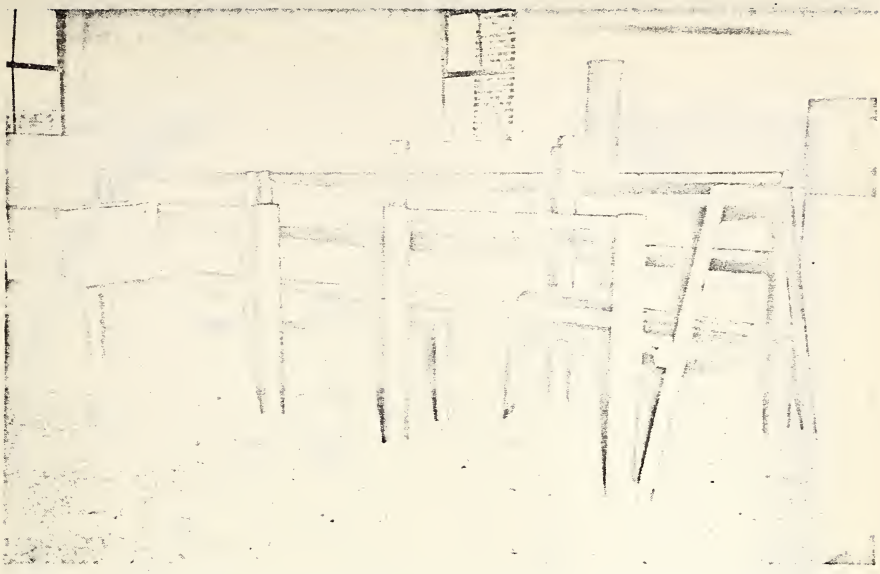

FIG. 5.—A convenient and safe semen collection chute in use at the LSU Dairy Improvement Center.

Collecting semen by massaging the ampullae is not a very satisfactory procedure, since the semen is often contaminated with urine and dirt. This method is used only where valuable bulls are unable to mount and serve the artificial vagina because of injury. Semen collected by this method has been observed to be inferior in quality and is usually less fertile than that collected by the artificial vagina. Special training is essential to obtain semen by massaging the ampullae.

Electro-ejaculation is a relatively new method of collecting semen from the bull. According to Dziuk *et al.* (11), it is possible by electrical stimulation to collect semen from some bulls that are unable or unwilling to serve an artificial vagina. Valuable animals that are old or crippled may still be used in spite of their physical handicap. Semen obtained by this method is of greater volume but of lower density. However, the total number of spermatozoa is comparable to that obtained with the artificial vagina. The electro-ejaculator has been used at this station on a limited basis with good results. Special skill is not required to operate the electro-ejaculator, and usually good results can be obtained by following the instructions of the manufacturer.

Evaluation of Semen

Much variation is observed in the quantity and quality of semen collected from different bulls and between ejaculates from the same bull. Each sample of semen must be carefully examined to determine

FIG. 6.—Microscopic view of spermatozoa.

its suitability for use in artificial insemination. Immediately after collection, each ejaculate should be examined to determine the general appearance, volume, per cent and rate of motility, concentration, and methylene blue reduction time. Several other tests, including pH, morphology, dead-alive staining, cold shock, incubation, and reaction rates, have been designed to measure semen quality, but they are used only to a limited extent because of the time, equipment, and skill required to make them. The methylene blue test and the determination of concentration are often omitted where artificial insemination is practiced in individual herds.

A normal semen sample is whitish-yellow with a milk-creamy consistency. Color is dependent to a large extent upon the number of spermatozoa present. A thick, cream-like sample is very high in concentration, whereas a thin, watery sample is low in density. Semen characteristics reported here are based upon some 20,000 ejaculates produced by 152 dairy and beef bulls during a nine-year period. These bulls were selected for use in artificial insemination and it is quite likely that the quality of this semen was better than that of the average of all bulls.

Semen volume per ejaculate ranges from 0.5 to 12 ml., with an average of 4.5 ml. Volume not only varies between breeds and bulls within a breed, but between ejaculates of an individual bull. As a rule, bulls of the larger breeds produce a greater volume of semen

per ejaculate, and volume usually increases with an increase in the age of the bull up to the fourth year.

The rate and percentage of forward movement of spermatozoa is observed microscopically. Progressive motility of spermatozoa ranges from 0 to 80 per cent, with an average of 50 per cent. Steps in determining progressive motility are as follows. (1) Warm a clean glass slide to approximately 100° F. A stage incubator should be used if available to keep the sample warm while it is being examined. (2) Mix semen by inverting the tube several times. Avoid vigorous agitation. (3) Place a small drop of semen on the slide with a sterile stirring rod. (4) Place a large drop of 3 per cent sodium citrate dihydrate on the slide next to the semen. (5) Mix the semen and citrate with one corner of a clean cover glass and cover the mixture with the cover glass. (6) Examine immediately, using the high power objective. Estimate the percentage of progressively motile spermatozoa to the nearest 10 per cent; that is, 40, 50, 60, 70, etc. At the same time, estimate the rate of movement. The rate ranges from 0 to 4, with 0 showing no forward movement; 1—movement is weak and very slow with little or no progressive motility; 2—shows a slow progressive movement; 3—movement is vigorous and more rapid than 2; 4—very rapid and vigorous movement. A sample containing less than 50 per cent motility or a rate of less than 3 is usually discarded.

The number of sperm per ml. normally ranges from approximately 300,000,000 to over 2,000,000,000, the average being about 1,200,000,000. There is much variation in the sperm count of semen produced by different bulls, with different ejaculates from an individual bull varying somewhat less. Concentration is very important in a large breeding association where maximum use is made of the semen. Density of the semen is somewhat less important in a private herd or in a small operation where a sample is used to breed a limited number of cows.

The conventional methylene blue reduction test, which is based upon concentration and activity of the spermatozoa, is not a good measure of potential fertility of the semen. However, the modified test which was devised by workers (7) at this station was found to be a reliable aid in measuring the initial spermatozoa activity and potential fertilizing capacity of semen. In semen that contains a high percentage of active spermatozoa, the oxygen is used at a more rapid rate than it is in a poor sample. This causes an excess of hydrogen, which combines with the methylene blue to form leuco-methylene blue, which is colorless.

Steps in making the modified methylene blue test are as follows. (1) Prepare a methylene blue solution by dissolving 50 mg. methylene blue chloride in 100 ml. of 3 per cent sodium citrate dihydrate. (2) After the concentration has been determined, place a sufficient

FIG. 7.—Evaluating semen for motility and concentration.

volume of semen to contain 240 million spermatozoa in a 10 x 75-mm. test tube and then add enough egg yolk-citrate extender to bring the total volume to 1 ml. and mix. (3) Add .1 ml. methylene blue solution (as prepared in step 1) and mix thoroughly. (4) Seal with ½ inch layer of mineral oil. (5) Place in water bath at 115° F. and observe the time required for the sample to lose its color. Methylene blue reduction time ranges from 4 to 12 minutes, with an average of 5 to 6 minutes. A sample requiring more than 9 minutes should be discarded.

No single rapid test has been devised that will accurately and reliably measure semen quality and predict its potential fertility. However, the following combination of tests and minimum standards are recommended for use in the evaluation of bull semen: (1) initial progressive motility of at least 50 per cent; (2) a concentration of at least 500 million spermatozoa per ml.; (3) a modified methylene blue time of 9 minutes or less. Previous breeding history of the bull is also an important factor to be considered.

Cooling and Storing Semen

Handling of semen according to proven methods is of utmost importance in a modern artificial breeding organization or in a small-scale operation on a private farm. Semen should be extended (diluted) and the cooling process started immediately after collection. According to Anderson and Seath (3), any delay in this step will result in a decrease in semen quality and fertility and an increase in bacterial growth. The extending fluid and semen should be of approximately the same temperature.

Much research has been conducted with the objective of extending the livability of sperm in storage and developing improved methods of shipping and handling semen in the field. In 1945, Mayer and Lasley (16) isolated a factor in egg yolk which increased the resistance of spermatozoa to "cold shock," reduced the time in which semen could be cooled, and extended the length of time that semen would remain motile in storage. Investigators (29) at the New York Station found the optimum temperature for long-time storage of semen to be between 34° and 41° F. If semen is to be stored at 34° F., it must be cooled at a very slow rate (9° F. per 20 minutes). Studies made at this station showed a marked reduction in the motility of semen stored at a temperature ranging from 28° to 40° F. as compared to samples stored at a more constant temperature of 38° to 40° F. Although spermatozoa will live slightly longer when stored at 34° F., the most practical temperature for storing semen used in artificial insemination is 38° to 40° F.

The cooling procedure used at the LSU Dairy Improvement Center is a modification of the system developed by the Cornell University workers. As soon as the semen is collected and brought into the laboratory, it is labeled, stoppered, and placed in a 250-ml. beaker containing approximately 150 ml. of water at 80° F. Volume, per cent progressive motility, and concentration are determined, and the methylene blue test is started within five minutes after the semen is collected. Samples that meet the minimum standards for motility and concentration are pre-extended at a ratio of 1:4 with egg yolk-citrate-sulfanilamide at 80° F. The test tube containing the partially extended semen is placed in a tall 250-ml. beaker containing 200 ml. of water at 70° F. Then the beaker containing the semen is placed in a walk-in refrigerator set at 40° F. By this means, the semen is cooled gradually to 40°-45° F. in 1 hour and 30 minutes without any danger of temperature shock. The final extension should be made in a refrigerator with extender which has been cooled to 40° F. If only a small amount of semen is needed, the final extension may be made before the semen is cooled.

Semen Extenders and Rate of Extension

The purpose of extending semen is to increase the volume in order that a larger number of cows may be bred to the most valuable sires. A good extender should not only increase the volume of semen, but it should also aid in the preservation of the spermatozoa by furnishing nutrients, reducing the effects of by-products produced by the sperm, and protecting the cells against cold shock and other adverse conditions. Many different fluids have been used for extending semen; however, only a limited number of them have proved to be satisfactory.

The two extenders most widely used in this country are the egg yolk-phosphate extender developed by Phillips and Lardy (21) in

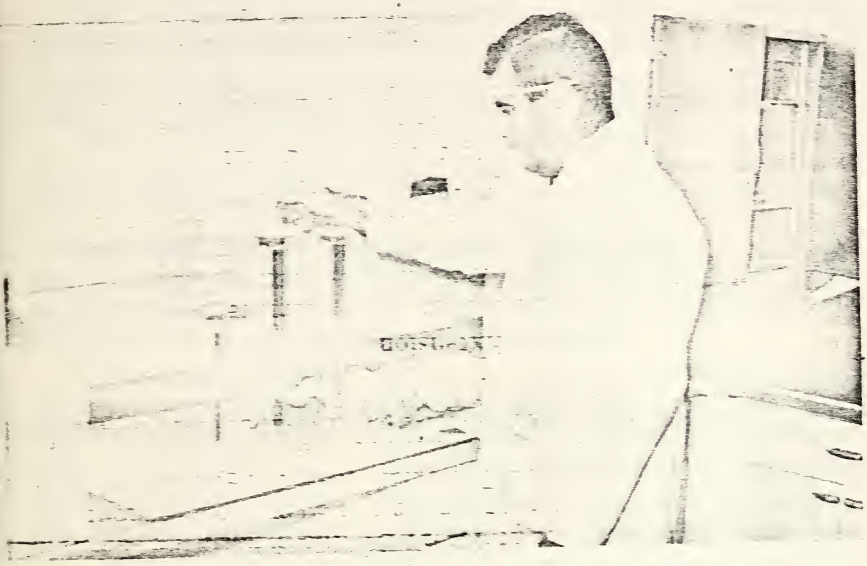

FIG. 8.—Preparation of egg yolk-citrate extender.

1940 and the egg yolk-citrate extender formulated by Salisbury, Fuller, and Willett (23) in 1941.

Although both of these extenders have been used with about equally good results, the yolk-citrate is usually preferred because the citrate disperses the fat globules in the egg yolk so that the spermatozoa can be seen more clearly under the microscope. In 1951, Thacker and Almquist (26) of the Pennsylvania Station found that the heating of pasteurized skim milk and pasteurized, homogenized milk brought about changes in these products which made them suitable for semen extenders. Spermatozoa remain motile in the boiled milk products for periods approximately equal to those extended in yolk-citrate. In preliminary field trials, these workers found that pasteurized, homogenized milk boiled for 10 minutes resulted in fertility equal to or slightly superior to that obtained from yolk-citrate extender containing 1,000 units per ml. each of penicillin and streptomycin. Shortly following this report, several artificial breeding associations tried various types of milk products as semen extenders. Wide variations in fertility were obtained from milk extenders by the various associations, and in many instances the fertility was lower than that obtained with yolk-citrate extender.

Investigators at this station compared pasteurized skim milk which was heated at 203° F. for 10 minutes with yolk-citrate. Sufficient amounts of penicillin and streptomycin were added to both extenders to give 500 units each per ml. of extended semen. Fertility, based on 30- to 60-day non-returns to first services, was 73.3 per

cent for the yolk-citrate and 70.4 per cent for the boiled skim milk. These differences were not significant. Storage motility after 72 hours was slightly, but not significantly, higher for the heated skim milk as compared to the yolk-citrate extender.

Milk extenders are more economical and much easier to prepare than either the yolk-phosphate or the yolk-citrate. The motility can be readily obtained in semen extended in skim milk, but it is difficult to see the spermatozoa under the microscope in whole milk.

There are several commercial semen extenders available through the artificial insemination supply houses. These preparations have been used successfully by individual dairymen and ranchers.

Egg Yolk-Citrate-Sulfanilamide Extender (Diluter)—To prepare egg yolk-sulfanilamide extender, use one part of egg yolk and three parts of citrate-sulfanilamide buffer and mix thoroughly. Directions for preparing these materials are given below.

Citrate-Sulfanilamide Buffer— (1) Weigh out 30 grams sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) and 4 grams sulfanilamide. (2) Place about 800 ml. of water distilled over glass in a wide-mouth flask and bring to a boil. (3) Add the citrate and sulfanilamide and mix thoroughly. (4) Allow the solution to cool to room temperature and then add enough water distilled over glass to bring the volume to 1,000 ml. (5) Mix thoroughly. (6) Store the citrate-sulfanilamide buffer in a brown jug in a dark place at room temperature until it is used. Light will destroy the sulfanilamide and turn the solution brown. It is desirable to make up a fresh supply of buffer every 2 to 3 weeks. Sulfanilamide will aid in the control of bacterial growth and may increase fertility but is not an essential part of the extender.

Egg Yolk—(1) Wash fresh eggs thoroughly in a warm detergent solution. Then rinse with warm tap water followed by a rinse in distilled water. (2) Rinse eggs in isopropyl alcohol or 70 per cent ethyl alcohol and let them dry. (3) Place a clean metal egg separator over a sterile graduated glass cylinder. (4) Break egg shell about midway of the egg on the edge of a clean beaker or jar. Transfer the yolk (yellow) from one-half of the shell to the other until all of the white is separated from the yolk. (5) Place the yolk in the separator which was prepared in step 3. Puncture the yolk membrane thoroughly with a clean glass rod and permit the yolk to run through the separator into the cylinder. (6) Discard the yolk membrane and repeat steps 4 and 5 until the desired volume of egg yolk is obtained. Several cylinders may be used if a large volume of extender is to be prepared. For best results, the extender should be made each time it is to be used and stored at 40° F.

Milk Extender—The procedure recommended by Pennsylvania workers (26) is as follows. (1) Obtain fresh, pasteurized, homogen-

ed milk or pasteurized skim milk from an established creamery. (2) Heat the milk in the top section of a pyrex glass double boiler to 203° F. Remove the thermometer, replace the lid, and continue heating for 10 minutes. (3) Remove milk from heat and allow it to cool to room temperature. (4) After cooling, pour the milk into another sterile container, leaving the scum in the boiler. It may be necessary to strain the milk through a sterile cloth. (5) Precautions: (a) any variation in the procedure may result in an unsatisfactory extender; (b) check the sperm motility during a seven-day storage period and try milk extender on a small scale before adopting it as a routine practice.

The Use of Antibiotics in Semen—All bull semen contains bacteria at the time of collection. The number of bacteria in semen collected with the artificial vagina ranges from 100 to more than 3,000,000 per ml., with an average of some 200,000 (2). Some of the forms most commonly found in semen are *diphteroids, bacilli, E. coli, pseudomonas, streptococci,* and *staphlococci.* Studies made at this station confirmed the reports of several other investigators showing no relation between number of bacteria present and fertility. However, there are certain forms of bacteria, including *pseudomonas pyocyaneus* and *hemolytic streptococci,* which are associated with low fertility. Almquist *et al.* (1) found that the addition of penicillin, streptomycin, or a combination of these materials increased the fertility of relatively infertile bulls as much as 25 per cent. Studies made at this station (9) with bulls of varying levels of fertility showed an increase of 16 per cent in fertility when 1,000 units of penicillin and streptomycin per ml. of extender were added. These materials not only increase fertility but control the bacterial flora, improve livability of spermatozoa, and act as metabolic depressors. In more recent studies 500 units of penicillin and 500 units of streptomycin per ml. of extended semen gave satisfactory results.

Preparation of Antibiotic Solutions. PENICILLIN—Add 19.5 ml. of 3 per cent sodium citrate buffer to a bottle containing 2,000,000 units of crystalline penicillin "G" and shake until all crystals are dissolved. This solution will remain active for seven days if stored at 40° F.

STREPTOMYCIN—Add 47.5 ml. of 3 per cent sodium citrate to a bottle containing five grams of streptomycin sulphate and shake until the material is completely dissolved. Streptomycin requires more vigorous shaking than penicillin. Streptomycin is more stable than penicillin and will remain active for 14 days if stored at 40° F. Both of these materials can be made up in smaller quantities if desired. Each of the above solutions contains 100,000 units per ml. Add one-half ml. of the penicillin solution and one-half ml. of the streptomycin solution per 100 ml. of extended semen. This will pro-

vide 500 units of penicillin and 500 units of streptomycin per ml. of extended semen.

Rate of Extending Semen—Semen contains varying numbers of spermatozoa at the time of collection. Several investigators, including Salisbury (22), have reported that high quality semen can be extended 1:100 without any decline in fertility. Many artificial breeding associations are obtaining good results with semen extended at a ratio of 1:100. If maximum use is to be made of the semen obtained from the most valuable bulls, it is much more logical to extend on the basis of numbers of motile spermatozoa. Studies made at this station (8) show a higher rate of fertility for semen with a high initial concentration as compared to samples with lower initial concentrations when the semen was extended by volume. However, when semen was extended on the basis of numbers of motile sperm, samples with a low initial concentration gave results equal to those with higher initial sperm counts.

An experiment was conducted at this station whereby semen was extended to contain 12, 6, and 2 million motile sperm per ml. Fertility based on 60- to 90-day non-returns to first services was 63.6, 64.5, and 58.2 per cent respectively for the three extension rates. New York workers (10) obtained a slight decline in fertility when semen was extended to contain 5 million motile sperm as compared to samples containing 10 million or more motile sperm per ml. Thus, it would appear that the threshold is somewhere between 5 and 6 million motile spermatozoa per ml. of extended semen or per insemination.

Where artificial insemination is being practiced on a small scale, such as on a private farm, there is no advantage in determining the concentration. Research has shown that good results can be obtained when the semen is extended according to volume. Many people make the mistake of extending semen at a very low ratio, such as 1:4 or 1:10. When semen is extended 1:25, the sperm will live longer than they will in a lower extension ratio and there is less chance of spreading disease such as *Vibrio fetus*. If the motility is 50 per cent or more, there is an adequate number of sperm present in semen extended 1:25 to result in maximum fertility unless the sample is extremely low in concentration.

Shipping and Handling Liquid Semen Under Field Conditions

In packing semen for shipment, the most important requirements are (a) adequate refrigeration, (b) insulation against outside temperature, (c) protection of semen tubes against breakage, and (d) insulation to prevent the semen from getting too cold. Missouri workers (14) found that an insulated corrugated cardboard box 6 inches x 6 inches x 12 inches was satisfactory for making overnight semen shipments. The prepared tubes of semen were wrapped in

several thicknesses of paper and enclosed in a "Jiffy Wrapper" with a can of ice. Then the insulated wrapper containing the semen and refrigerant were inserted into the cardboard box and sealed.

A modification of the Missouri packaging procedure is used at this station. Steps used in packaging semen are: (1) After the semen has been evaluated, extended, and cooled to 40° F., it is poured in properly labeled plastic tubes and capped. The tubes are wrapped in four thicknesses of paper towel and then a piece of corrugated cardboard 4 inches x 6 inches is folded around the bundle of tubes and secured with a rubber band. (2) The tubes enclosed in the cardboard are placed between two number two cans of ice in a pre-cooled quart size "Jiffy Bag." (3) After the bag has been folded at the top, it is inserted in a pre-cooled 5 inches x 5 inches x 11 inches corrugated cardboard box insulated with a "Jiffy Liner," and the box sealed. Semen packaged in this manner will maintain a constant temperature of 38° to 40° F. for 18 hours or longer.

Handling semen properly is one of the most important phases of the breeding technician's work. In order for the technician to obtain the best breeding results, the semen must be stored and handled according to approved procedures. There are several commercial kits on the market which will keep the semen at the desired temperature of 38° to 40° F. if they are properly iced. The ice chest in any kit that is used should be equipped with a thermometer so that the technician can check at regular intervals to make sure that the semen is being maintained at the desired temperature at all times.

The following procedure for handling semen has been found to give good results: (1) Upon arrival of the semen, open the package and check the cans to see if they still contain ice; if so, the temperature of the semen is very likely to be under 45° F. (2) If all the ice in the can is melted, then the temperature should be determined by inserting a thermometer between the tubes of semen and letting it remain for five minutes. The tubes of semen and cans should be placed back in the bag and the carton should be closed during this period. If the temperature is 45° F. or lower, the tubes of semen may be placed in the ice chest, which should be between 38° and 42° F. However, if the semen arrives at a temperature above 45° F., the tubes should be placed in a glass of water near the temperature of the semen and cooled gradually at a rate of 1° F. every two minutes. (See cooling procedure in another section of this bulletin.) (3) It is a good practice to examine the semen to determine the motility each day. (4) Semen more than three days old and samples which have been exposed to high or low temperatures should always be examined before using. (5) Check the temperature of the ice chest at regular intervals; the temperature should be betwen 38° and 42° for best results. (6) Never allow water to remain in the metal tubes in the ice chest. Air around the tubes of semen aids in preventing cold

shock. (7) Always use coil springs in metal tubes to push up tubes and prevent breakage. (8) Keep semen clean and avoid excessive shaking.

Tubes of semen may be stored in a refrigerator. The temperature of the semen will remain more constant if the tube is placed in a beaker or glass containing enough water to cover the lower half. A thermometer should be placed in the glass so that the temperature can be checked from time to time.

A wide-mouth thermos bottle can be used in place of an ice chest in a small artificial insemination operation, such as on a private farm. Several holes can be bored in the cork to provide a place for carrying the semen tubes. Water in the thermos should be 38° F. If the thermos is used, a small metal chest similar to the regular inseminating kit will be needed to carry the tubes, syringes, and other inseminating supplies.

Processing, Storing, and Using Frozen Semen

The freezing and long-time storage of semen offers a new and useful tool to purebred dairy and beef breeders as well as commercial operators. These farmers with genetically superior animals will be able to increase the usefulness of their sires and follow a more intelligent mating system through the proper application of a semen freezing program. In Louisiana, as in most subtropical and tropical regions, many sires experience low semen quality during the hot and humid summer months. This degenerative change in semen production may last into the fall and early winter months. In particular instances, the effects of hot weather may be severe enough to cause permanent damage to the reproductive organs of a bull; however, in most cases those animals that are affected recover by early spring.

During the periods of low quality semen production, the fertility level will normally decrease in proportion to the extent the semen quality has been affected. Thus, the possibility of a breeder's freezing semen during the spring when semen quality is high and storing it for periods up to a year or longer will in many instances prove to be an economical and sound approach to a planned mating program. The following information concerning frozen semen is intended to familiarize the breeder with the more important technical aspects of freezing, storing, and using frozen semen. Certain phases, such as storing, may be carried out by the breeder, but only those well trained in the procedure of semen collection and processing should undertake the actual freezing of semen. In some instances the breeder will be able to contract with a commercial or state organization for the collecting and freezing, and in some cases even the storing of the frozen semen.

The information presented is in part based on knowledge gained from practical investigations with frozen semen conducted at LSU and from research work at the New Jersey Experiment Station (17) and breeding associations throughout the country.

Preparation of Extender and Freezing of Semen

Semen for freezing can be extended in either egg yolk-citrate or milk extender. Egg yolk-citrate extender has been used primarily in Louisiana for both laboratory and field studies.

Preparation of Egg Yolk-Citrate-Glycerol Extender—Prepare a 3 per cent sodium citrate dihydrate buffer solution. This solution may be kept several weeks under refrigeration. To the buffer solution (warm to 30° C. prior to using), add egg yolk at the ratio of 1:4 and 1,000 micrograms of streptomycin sulfate per ml. of extender prepared; mix thoroughly. This portion of the extender will be referred to as solution A.

In another flask prepare a solution containing 66 per cent buffer solution, 20 per cent egg yolk, and 14 per cent glycerol; mix thoroughly. This portion of the extender will be referred to as solution B.

Extending, Cooling, and Equilibration—Following microscopic evaluation, extend the semen sample in solution A at 30° C. Semen used for freezing should contain a minimum of 50 per cent motile spermatozoa. Extend semen that is of average concentration 1:20. When an accurate determination of the concentration can be made, extend at the rate of 30 million live sperm per ml. The extended semen is then cooled slowly at 5° C. over a period of 2 to 3 hours. To accomplish this, put the tube of extended semen in a tall 250-ml. beaker approximately three quarters full of tap water at 30° C. Next, place in a refrigerator or walk-in cooler where the temperature is controlled at 5° C. After the semen is cooled to 5° C., add an equal volume of solution B at 5° C. in five equal aliquots over a period of 30 minutes. Allow an 18-hour equilibration period before starting the freezing process.

Fig. 9.—Dry ice and alcohol method of freezing semen.

The final composition after equal amounts of solutions A and B are added will be 20 per cent egg yolk, 2.2 per cent sodium citrate dihydrate, 7 per cent glycerol, and 500 micrograms of streptomycin sulphate per ml. If semen is extended by volume, the final extension

rate will be 1:40, or 15 million live spermatozoa per ml. when extended according to spermatozoa count.

Tapered pyrex glass ampoules have been found to be most satisfactory for packaging semen into individual 1-ml. inseminates. For permanent identification, use a glass-marking ink or alcohol resistant tape label. Dispense 1-ml. portions of the glycerolated semen into glass ampoules. For filling a small number of ampoules, a 20-ml. syringe is convenient; with a large number of vials, an automatic syringe unit is highly desirable. Use a 14-gauge needle for best results on both types of syringes. Seal ampoules with an oxygen-gas burner. The ampoules are placed in racks and transferred to an alcohol bath at 5° C. The freezing rate is controlled by the addition of dry ice at a rate that will decrease the temperature of the alcohol bath 0.8° C. per minute between +5° C. and —15° C. and 3 to 4° C. per minute between —15° C. and —79° C.

Storage of Frozen Semen

After they have reached —79° C., the ampoules are placed in a tray containing alcohol (—79° C.) and transferred to storage facilities. The storage temperature must be maintained at —75° C. or colder. There are several types and sizes of storage containers available. The combination mechanical-dry ice chest shown in Figure 10 has proved to be economical and suitable for long-time storage of semen. The arrangement of a series of three drawers, each containing five trays, allows for easy accessibility to the semen and for a maximum capacity of 1,260 ampoules. The three drawers are housed in a perforated metal ice-retaining wall. Guides fastened to the walls allow the drawers to be pulled up with quick access to any one of the five trays. Cracked dry ice is placed between the metal retaining wall and the styrene insert and on top of the drawers. Studies made at this station with the chest shown in Figure 10 indicate that dry ice must be added at 14-day intervals. The average daily consumption of dry ice was 4.26 pounds, and the temperature in the trays ranged from

Fig. 10.—Mechanical-dry ice frozen semen storage chest.

—77.5° to —78.5° C. Dry ice should not be allowed to drop below two-thirds capacity. This system of semen storage is particularly advantageous over mechanical refrigeration where power failures are frequent.

Several companies have mechanical freezers on the market for frozen semen storage. These units vary in size with storage capacities ranging from 1,000 ampoules upwards to 20,000. Many of the mechanical boxes have safety features, such as the automatic release of CO_2 in case of power or mechanical failure.

A dry ice storage chest as shown in Figure 11 is suitable for storage of 300 to 400 ampoules. Dry ice is placed around a retaining wall of perforated metal and in the center on top of the stored semen. A series of trays is used for convenient handling and accessibility to the semen.

Wide-mouth thermos bottles are also used for storage of small quantities of frozen semen. One-gallon vacuum thermos jars will hold up to 150 ampoules. To separate the ampoules from the dry ice, a wire-mesh divider is placed in the center of the jar. The dry ice is on one side of the divider and the ampoules are placed on the other side. Before placing the ampoules in the thermos, add one quart of alcohol and cool down with dry ice to.—79° C. The thermos jars must be re-iced every two days when kept at room tempera-

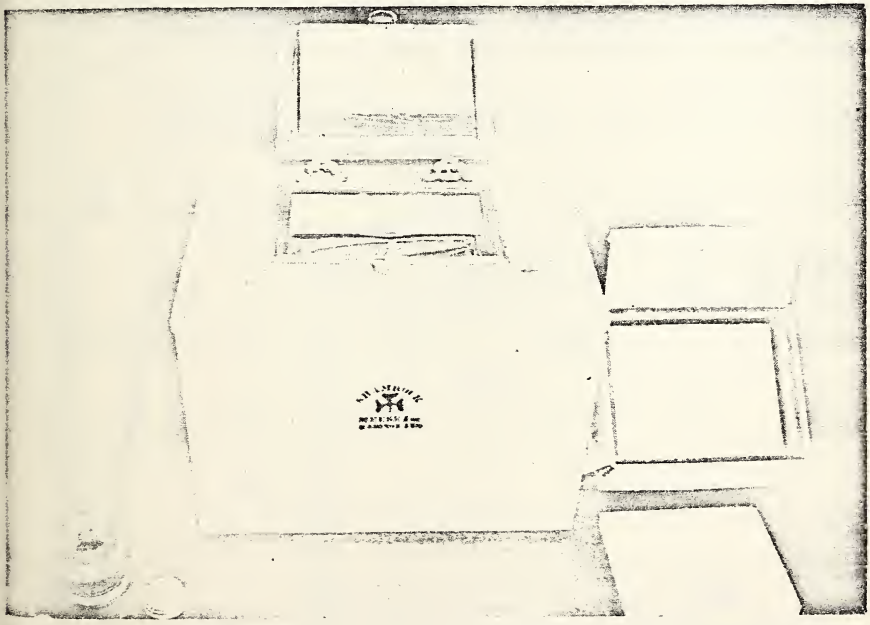

FIG. 11.—Equipment used for storing and shipping frozen semen. Shown are: wide-mouth pint thermos bottle, storage chest (400-ampoule capacity), and styrene shipping container.

ture. This interval can be extended when the jars are maintained in a deep freeze.

A wide-mouth pint thermos bottle is convenient for carrying semen to the field. Add 1/3 pint of alcohol and cool slowly to —79° C. with finely cracked dry ice. Place ampoules in cooled mixture and fill bottle with dry ice.

The styrene shipping container shown in Figure 11 has proved to be practical for shipping frozen semen. Fill the box 1/3 full of cracked dry ice. Place ampoules in a container such as a pint paint can partially filled with alcohol and secure lid tightly. The container of ampoules is then placed in the center of the dry ice. Complete filling box with dry ice. Frozen semen packaged in this manner may be shipped by air or bus.

A more recent development for semen storage is the liquid nitrogen refrigerator shown in Figure 12. The refrigerator has a capacity of 33,000 ampoules of semen and is maintained at a temperature of —196° C. Daily consumption of liquid nitrogen is approximately 7 liters, with the temperature holding time (without refilling) being about two months.

The liquid nitrogen central storage refrigerator is constructed of stainless steel, with all joints having been welded to give added strength and to insure long-lasting vacuum in the wall space. Stor-

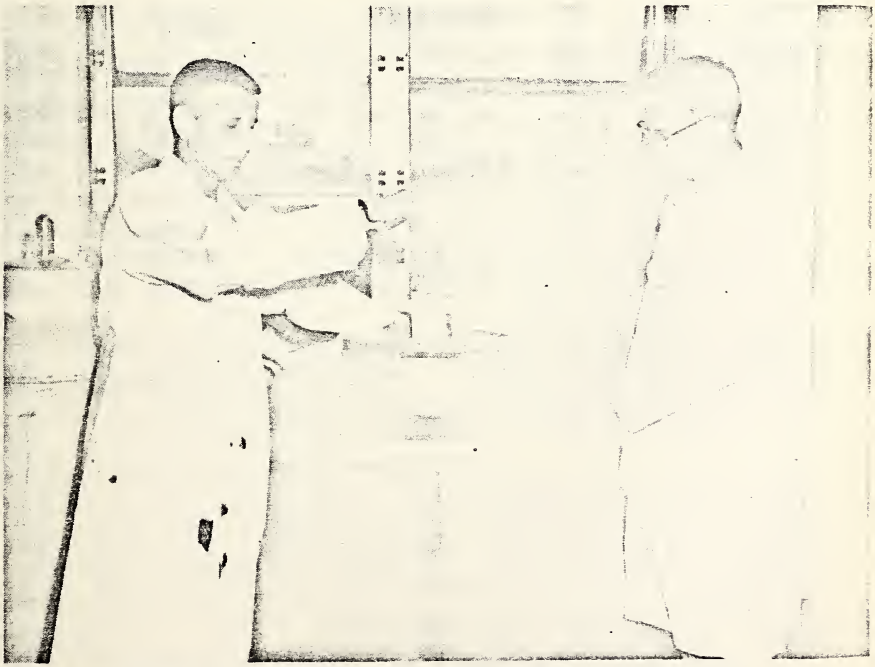

FIG. 12.—Liquid nitrogen central storage refrigerator.

...ge space consists of two circular trays, each of which is divided into pie-shaped sections. The bottom tray has six usable sections but the top has only five since one section of the top tray is left open to give access to the bottom tray. Each tray can be rotated separately by means of a handwheel.

Ampoules of semen are attached to metal strips referred to as canes or racks. Each cane holds six ampoules and is marked at the top with the code number of the bull. Canes of semen are packed in cartons 2½" x 2½" x 11", with the top left open. Each carton holds 20 canes, or 120 ampoules. Each pie-shaped section of the trays holds 25 cartons. An ampoule, cane, or carton of semen can be removed without exposing any of the others to the outside temperature.

There are many other types of storage boxes and containers on the market. Some of these products have been tried experimentally and have proved satisfactory under certain conditions. The major points to consider when purchasing such equipment are the reliability of the manufacturer, electric and dry ice consumption, accessibility of the ampoules, and a storage temperature of —75° C. or colder.

Care and Use of Frozen Semen in the Field

To insure high fertility with frozen semen, the exact procedure for freezing, storing and field use must be followed. The frozen semen must be maintained at —75° C. or lower at all times prior to its use. The liquid nitrogen field refrigerator shown in Figure 13 is being used throughout the United States and has proved to be entirely satisfactory for a technician inseminating 1,000 to 3,000 cows a year. Construction is similar to that of the central storage container. The portable field refrigerator holds 504 ampoules of semen, which is maintained at —196° C. This refrigerator uses about 1 1/3 liters of nitrogen daily and must be refilled every 14 to 21 days. Semen is stored on canes in metal canisters or baskets. Each of the six canisters holds 14 canes, or 84 ampoules, of semen. A single ampoule of semen can be removed without exposing any of the other semen to outside temperature.

Investigations at this and other stations show that semen stored in liquid nitrogen has a slightly higher percentage of motile sperm after thawing as compared to storage in dry ice. Most investigators also agree that there is some improvement in fertility with storage temperatures of —196° C. Liquid nitrogen storage equipment facilitates the handling of semen both by the technician and laboratory personnel, since it is well below the critical temperature.

To thaw frozen semen, place the ampoule in a pint jar of water adjusted to 5° C. After thawing, blot dry with paper towel and scratch the ampoule at the constriction with a small file. Place a

FIG. 13.—Liquid nitrogen field refrigerator.

paper towel over the tip of the ampoule and snap off the top. The opening at the break is of sufficient size to allow the insemination tube to pass.

In order to compare fresh and frozen semen under Louisiana conditions, a field trial (15) involving 6 bulls and 18 technicians was conducted. A portion of the semen from each bull was extended to contain 10 million live sperm per ml. and used in the liquid state over a three-day period. The remainder of each sample was extended to contain 15 million live sperm per ml. and frozen. Antibiotics were added to both extenders at the rate of 500 units of streptomycin and penicillin per ml. of extended liquid semen and 500 micrograms of streptomycin per ml. of extended frozen semen. A total of 119 cows was bred with fresh semen and 194 with frozen semen. Fertility, based on 60- to 90-day non-returns to first services was 68.9 for the fresh and 68.0 per cent for the frozen semen. Several artificial breeding associations in the United States, Canada, and England have reported equally good results from liquid and frozen semen. However, better trained and more closely supervised technicians are required when frozen semen is used.

Suggestions and Precautions Regarding Frozen Semen

1. Use semen of high quality for freezing.
2. Second ejaculates normally freeze better than first ejaculates.

3. Semen samples from the same bull may vary as to percentage of recovery after freezing.
4. Semen in storage shows a gradual decline in motility even at −78° C.; however, a much greater decline is noticed at storage temperatures ranging from −65° to−70° C.
5. Cool down storage containers at least 24 hours prior to adding semen.
6. When transferring frozen semen, do it as quickly as possible. A slight change in temperature may affect the future storage qualities of the semen.
7. Maintain an adequate supply of dry ice in storage containers to insure proper storage temperature.
8. Do not refreeze semen, even if only partially thawed.

Insemination of the Cow and Determination of Pregnancy

Artificial insemination is a skill which can only be mastered by training and experience. The length of time required for a breeding technician to reach maximum efficiency varies from 2 to 6 months depending upon the interest, background, and ability of the individual. A general knowledge of the structure and functions of the reproductive organs is essential.

Methods of Insemination

Three basic methods of artificial insemination are vaginal, shallow cervical (speculum), and deep cervical or intra-uterine. Each of these methods is discussed below.

Vaginal Method—In the vaginal method of insemination, the semen is deposited in the anterior portion of the vagina, just below the cervix, with the aid of an inseminating tube and syringe. This procedure is very simple and requires a minimum amount of equipment, but it has not proved to give a satisfactory conception rate. Thus, it has little or no place in an organized artificial breeding program.

Shallow Cervical Method (Speculum)—In the shallow cervical method of insemination, the semen is deposited in the opening of the cervix with the use of a glass speculum and a plastic or glass inseminating tube and syringe. After the speculum has been placed in the vagina, the cervix can be located with the aid of a small flashlight or headlight. The shallow cervical or speculum method of insemination was used extensively in former years with a fair degree of success. However, with the rapid development of artificial insemination in the United States, the speculum technique has been replaced by the more efficient intra-uterine method. In research at the Missouri station (28) the intra-uterine technique resulted in a 10 to 12 per cent higher breeding efficiency than the speculum method.

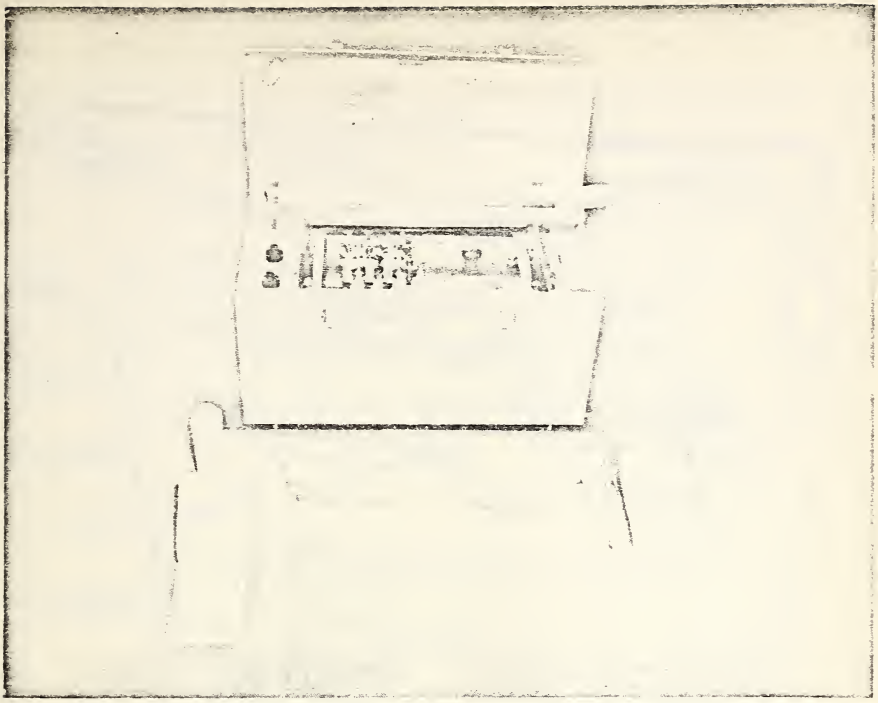

FIG. 14.—Equipment and supplies used in inseminating the cow.

Deep Cervical or Intra-Uterine Method—This method of insemination has given very good results and is being used almost exclusively in the United States and several of the European countries. Equipment and supplies used in this method are as follows: inseminating kit (including ice chest), 5-mm. disposable plastic inseminating tubes, 1-ml. plastic or rubber disposable syringes, 21-inch latex rubber glove, fabric or rubber sleeve, good quality cake soap such as Ivory, good quality disinfectant such as Creolin, talcum powder, paper towels or absorbent cotton, rubber boots or rubber overshoes, pail and brush for washing and disinfecting gloves and boots, notebook cover (three-ring notebook for individual farmer's herd records), indelible pencil, Purebred Dairy Cattle Association approved breeding receipts, and microscope (slides and cover glasses).

Steps used in the deep cervical or intra-uterine technique are as follows:

1. *Identification of the Cow to Be Inseminated*—This does not usually present a problem if the owner or a member of his family is available to point out the animal to be bred. All members should be provided with identification tags to be used on the cow when they have to be away from the farm. The cow to be inseminated should be placed in a stall or small lot when she is found in heat. This will

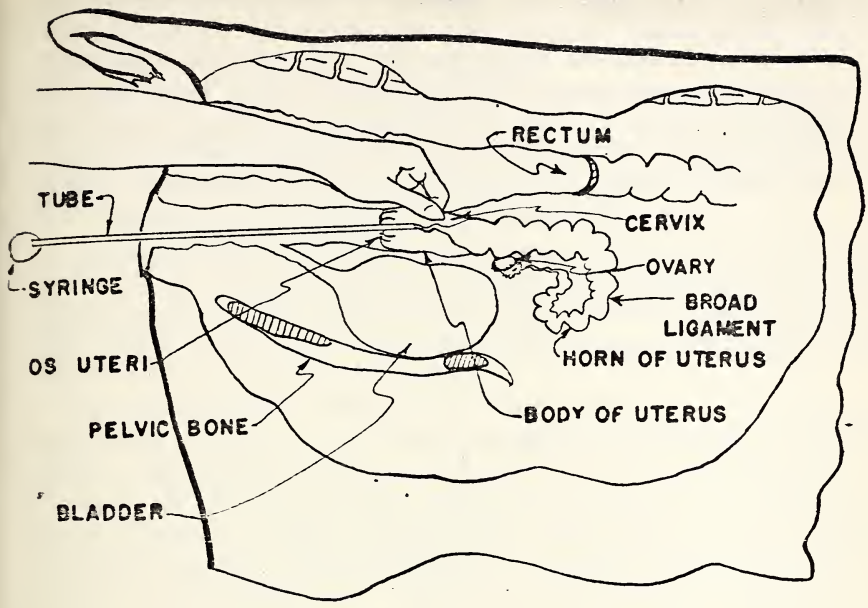

FIG. 15.—Diagrammatic drawing showing the technique used in making the deep cervical insemination.

avoid excitement of the cow just prior to insemination and eliminate unnecessary delay of the technician. All grade cows must be identified by ear tag or some other permanent number such as a neck chain or tattoo. Purebred cows must be identified by checking registration papers against the color markings or tattoo of the cow. If the papers are not available, the tattoo number or a sketch showing the color markings should be used for temporary identification. It is the responsibility of the owner to have the papers or a sketch of the color markings available when the breeding technician arrives. (See Purebred Dairy Cattle Association rules for complete information on inseminating purebred cows.)

2. *Restrain the Cow*—The cow should be properly restrained in a stanchion or tie stall before insemination is attempted. It is very important to handle the cow gently prior to, during, and immediately after insemination. Excitement or rough treatment will reduce the rate of conception.

3. *Clean the Vulva*—Clean and dry the vulva and surrounding area with a paper towel or absorbent cotton.

4. *Prepare Inseminating Tube and Draw Up Semen*—(a) Remove inseminating tube from metal speculum, being careful not to touch it except near the end where the syringe is to be attached. (b) Obtain a rubber or plastic syringe from the sterile glass jar and attach

it to the tube. (c) Remove tube of semen to be used from ice chest and check label carefully for name and number of bull. (d) Mix semen by gently inverting the tube several times. Avoid vigorous shaking of the semen, as this will injure the sperm. (e) Remove the cap and draw 1 ml. of semen into the inseminating tube. Replace the cap, put the tube of semen back in the ice chest and replace the snap-tite rubber stopper. (f) Place the prepared tube and syringe on hooks in the lid of the kit or hold it in the mouth. If hooks are used for this purpose, they should be cleaned daily.

5. *Put on Glove and Sleeve*—Dry your left hand and arm and dust with powder. Work the glove on gently, making sure that the finger tips fit tightly. Take a bar of soap in the left hand and a brush from the pail of water and lather the entire glove. Put several paper towels in your pocket for later use.

6. *Locate Cervix Through the Rectal Wall*—As the technician approaches the cow, he should speak to her and pet or rub her gently. The fingers of the gloved hand are gently inserted into the rectum with the hand being cupped to permit air to enter. If the hand is held in a cupped position for a short time, the cow will often clean her rectum. Occasionally it is necessary to remove the manure with the hand. Many experienced technicians inseminate cows without emptying the rectum. Locate the cervix by feeling downward through the rectal wall. This may be difficult at first, but it can be located readily after a few practice periods. The cervix should be pushed to the right and held gently between the thumb and fingers. Wipe the vulva with paper towel, making sure that the outer surface is clean and dry.

7. *Inseminating*—The kit should be placed so that the inseminating tube can be reached with the free hand. If the tube is held in the mouth, it is not necessary to place the kit in reaching distance. Insert the prepared inseminating tube 3 to 4 inches into the vagina with the syringe end of the tube tilted downward. After the tube passes over the elevated border and becomes more nearly free, it should be leveled and gently passed through the vagina until the cervix is reached. An inexperienced person will usually have difficulty in locating the mouth or opening of the cervix. This can be accomplished by holding the cervix between the second and third fingers and placing the thumb over the opening. Bring the inseminating tube forward until it touches the thumb, then remove the thumb and insert the tube. The cervix consists of several folds which make it difficult for the untrained person to pass a tube through the crooked canal. However, this can be accomplished by manipulating the cervix while gentle pressure is exerted on the tube. Probing or thrusting of the tube should be avoided. Deposit the semen near the mid-point of the cervix. Withdraw the tube and remove the glove hand from the rectum.

Studies (19) at this station indicate that no increase in breed- efficiency can be expected by depositing the semen beyond the ldle of the cervix. When the inseminating tube is passed only f way through the cervix, there is less chance of infection and in e the cow is with calf, pregnancy is not likely to be interrupted. wever, if an inseminating tube is passed through the cervix into uterus of a pregnant cow, abortion will usually occur.

8. *Clean and Disinfect Glove*—Add disinfectant to the pail of ter and wash glove with a bottle or scrub brush. Water from a e may be used to rinse the glove before it is washed in the disin- ting solution. Dry the glove thoroughly inside and out and powder ith talcum. Fold the glove, pressing out all air possible, and place a small box or wrap it in paper.

9. *Breeding Records*—Write breeding receipt and post farmer's ividual herd record and the barn breeding record. Give the orig- l copy of the receipt to the owner and collect breeding fee, if any.

10. *Tagging the Cow*—If the cow is a grade, put a tag in her ear ess she already has an ear tag or some other identification num- such as a tattoo or neck chain. The tag should not be placed in ear until at least five minutes after the insemination is completed e any excitement will delay sperm movement and may reduce chance of conception.

11. *Sanitary Precautions*—Place the used tube and syringe in the y or speculum provided for this purpose. Put all used towels in a sh can and leave the barn clean and orderly. When you are ready eave, take your kit and pail of disinfectant to the car. Wash your ts or overshoes thoroughly before emptying the disinfecting solu- . This practice will lessen the chance of your spreading diseases ween farms and will make a good impression on the dairyman. e disinfectant should be emptied on the road or some other place ere nothing is growing, as it may kill grass or other vegetation.

gnancy Determination

The ability to diagnose pregnancy is of prime importance for a eding technician. Routine pregnancy determination is not the hnician's job or responsibility. However, it is of utmost impor- ce for him to examine cows that come in heat several months er insemination or in cases where the breeding history is not own. Records at this station indicate that approximately 5 per t of the cows show signs of heat during pregnancy. Failure to re- n to heat after breeding is a reliable indication of pregnancy in althy cows but may be misleading where breeding difficulties are ng experienced. Results of a study at the Wisconsin station (5) licate that about 80 per cent of the cows that do not come back in at within 45 days after insemination are pregnant. More than 95 r cent of the cows that did not return to heat within 105 days

were found to be pregnant. Postestrus bleeding, which may be seen in about 80 per cent of the heifers and 60 per cent of the cows, does not indicate whether the animal conceived.

Wisnicky (31) described a method whereby pregnancy can be diagnosed as early as 35 days by palpating the amnionic vesicle in the horn of the uterus. The gloved hand, which has been lubricated with soap and water, is inserted into the rectum of the cow with the palm turned downward over the coiled horns of the uterus. After the uterus is located, it is picked up and held in the hollow of the hand. With the uterus in this position, the middle finger should lie over the place where the horns come together. The extended finger is pushed down between the horns until it can be hooked under the web that connects them. By lifting upward and backward, the horns can be uncoiled so that the entire length of each may be palpated between the thumb and fingers. The fetus cannot be detected in early pregnancy. It is in a sac-like structure surrounded by fluid which is called an amnionic vesicle. Palpation is made for the presence of this vesicle, which will appear as a small, turgid, slightly oblong, balloon-like structure. As the fingers are moved backward along the horn with gentle pressure being applied, the vesicle can be felt to slip away as an attempt is made to palpate it. The slippery feeling is unlike anything else in the horn and is a rather definite indication of pregnancy. Usually, the amnionic vesicle will be found in the horn on the same side as the ovary in which the corpus luteum is present. However, investigations made at this station indicate that the fetus is occasionally located in the horn on the opposite side from the ovary in which the corpus luteum is found. In the case of twins, there may be two vesicles in one horn or one in each horn.

At 35 days, the vesicle is balloon-like and is about 1¼ inches in diameter. The vesicle grows and elongates rapidly between the thirty-fifth and forty-ninth days. After the seventh week of pregnancy, the slipperiness disappears rapidly and the pressure within the vesicle decreases. The cervical seal forms some 30 to 40 days after pregnancy is initiated. By 60 days, the fetus is some 2¾ inches long and is surrounded by a large amount of fluid, with the pregnant horn being almost twice its normal size. The fetal membranes may be felt to make sure that the uterine enlargement is due to pregnancy rather than metritis or tumors. This test consists of picking up the uterus and its fetal membranes, first allowing the fetal membranes to slip between the thumb and index finger and then the uterine wall. If only one tissue is felt to slip through the fingers, it will be the uterine wall, indicating that the fetal sac is not present.

According to Fincher (12), the cotyledons can be felt and the fetus can be palpated as early as the third or fourth month of pregnancy. After the fifth or sixth month, the fetus drops forward and downward and may be beyond reach. However, at this stage the

middle uterine arteries within the broad ligaments just back of the ovaries, alongside the cervix and base of the uterine horns are of extreme value in diagnosing pregnancy. The arteries will be ¼ to ½ inch in diameter and the blood can be felt to "swish" through the fingers. It is not a sharply defined throb, as in the arteries of a non-pregnant cow or during the early stages of pregnancy.

"Bumping" or palpating the calf through the flank of the cow is usually possible after the seventh or eighth month of pregnancy (25). By pressing the hand strongly and several times in succession against the right flank, about eight inches in front of the stifle and slightly below it, the movement of the fetus can usually be felt. Since this method cannot be used until the latter part of pregnancy, it is of little or no value in artificial insemination or in an efficient dairy operation.

Early pregnancy diagnosis is of prime importance. Pregnancy diagnosis can be learned only by practice and experience. The technician or herdsman can educate his finger tips by examining cows at every opportunity and relating his findings to breeding records.

The age of premature or aborted calves may be estimated from the following information reported by the Minnesota station (30). At four months of age pigmentation of the hair follicles may be seen, and the fetus is about 10 inches long and weighs about 1¾ pounds. Hair appears around the eyes at five months and the fetus weighs about six pounds. The body of the calf is covered with hair at eight months.

Fertility of Cattle

Fertility in the cow may be defined as the ability to produce fertilizable ova or eggs and to provide a tubular and uterine environment satisfactory for the processes of fertilization, implantation, embryonic and fetal development, and parturition. In the bull, fertility may be defined as the ability to produce large numbers of spermatozoa which are capable of fertilizing eggs and initiating and maintaining the processes of cell division necesary to the development of the embryo. It further implies that the bull must be physically capable of depositing spermatozoa in the female reproductive tract or in the artificial vagina when he is used in artificial breeding. Thus, it can be readily seen that fertility depends upon a series of complicated physiological processes in both sexes and that it is possible for many things to happen between the time a cow is bred or inseminated and the birth of a fully developed normal calf. However, before discussing the factors which affect fertility, the different measures of fertility should be considered.

Measures of Fertility

There are many measures of fertility. It can be measured in the cow in terms of the number of services or breedings required per

conception or per calf. Dairy cows require an average of about 1.6 services per conception. Other measures of fertility are calving interval, average months per calf during the reproductive life of a cow, and the number of calves per 100 females bred. It is highly desirable for a cow to produce a calf every 12 months. For range beef cattle, calf crops average from 50 to 70 per cent. Herds of well-fed and well-managed beef cattle will generally approach 90 per cent calf crops. It should be appreciated that any measure of fertility for the individual cow is subject to a great amount of error and variation.

For bulls used in artificial breeding, fertility is usually expressed as the percentage of cows not returning for service within an arbitrarily established period of from 30 to 180 days, the percentage of 60- to 90-day non-returns to first services being the most commonly used measure. It has been found that the percentage of 60- to 90-day non-returns to first services closely approximates actual pregnancy, having an error of only about 5 or 6 per cent. Fertility levels of individual bulls calculated on this basis vary from 30 to 85 per cent, averaging about 70 per cent. Since artificial breeding provides an opportunity to breed one bull to many cows, the level of fertility of an individual bull can be determined accurately.

Factors Influencing Fertility

Since neither the nature nor the relative importance of many of the factors influencing fertility of cattle in Louisiana was known, a series of studies was begun in 1951. In these studies, the factors were grouped into two major categories; namely, heredity and environment. It is very important to know what fraction of the differences in the fertility among cows is due to heredity and what part is due to environment before wise decisions can be made in any selecting and breeding program for improving fertility.

Heredity—Analyses of the breeding and calving records of the LSU Holstein herd for the period 1931 through 1946 revealed that there were 370 daughters of 15 sires that required an average of 1.71 services per conception, ranging from a low of 1.13 to a 2.23 services per conception. Thus, the daughters of certain sires were better breeders or had higher fertility than the daughters of other sires, indicating some hereditary basis for fertility. Statistical analyses of the data, however, showed that services per conception in these daughters were only 10.6 per cent heritable with a repeatability of 9.8 per cent. In other words, 89.4 per cent of the differences in the fertility of the cows were due to environmental factors and only 10.6 per cent of the differences were due to heredity. The estimate of 10.6 per cent for the heritability of services per conception agrees very closely with the estimates reported by other investigators.

Under present conditions, therefore, it seems that it would hardly pay to select for fertility upon the grounds of heredity using the measures of fertility now available, when there are more strongly heritable factors, such as those for milk, fat, and type, that might suffer in the selection. The greatest improvement in fertility could be made through working with some of the environmental factors which affect fertility. However, it should be emphasized that these results do not mean that there is no heritable tendency for low fertility or sterility, but that heredity accounts for only a small part of the over-all infertility problem. There have been two different genes associated with infertility, but apparently the frequency of these genes is very low in most herds and in the over-all cattle population.

Another aspect of this investigation which should be discussed briefly is the repeatability of 9.7 per cent for services per conception. Repeatability may be defined as the similarity of successive records of the same individual. An estimate of repeatability tells us how much we can trust a past record or records of an animal as an indication of its future performance. The estimate of 9.7 per cent is very low. Therefore, the number of services required per conception for a cow this year is a rather poor indication of how many services she will require next year. These results also are contradictory to the common belief: "Once a hard breeder, always a hard breeder." A cow might require six services for conception this year and settle on the first service next year. One should not be too hasty in culling a cow on the basis of her fertility during a single year. However, it is realized one must weigh the inherent producing ability of the cow in question against the economy of keeping her dry for any extended period of time.

Environment—Since heredity seemed to play such a small part in over-all fertility of dairy cattle, investigations were initiated to obtain information on the environmental factors affecting fertility of dairy cows in Louisiana. These investigations were done in the Louisiana State University dairy herd and in 84 other herds in different sections of Louisiana. A total of 383 "problem" breeding cows were examined. The environmental factors considered were as follows: time of postpartum service, timing of service during the heat period, age of cow, seasonal effects, diseases, physiological disorders, and general feeding and management practices.

Time of Postpartum Service—Since previous studies had indicated that the highest fertility was obtained when cows were bred 60 to 90 days postpartum (after calving), this practice was recommended from the outset (1947) in the Louisiana Artificial Breeding Program. These recommendations were based not only on the fertility results but also on the fact that certain changes must take

place in the uterus of the cow before conception can occur readily. The fluids present in the uterus after calving must be expelled or be reabsorbed into the blood stream of the cow, and the uterus must return to its normal size and healthy condition. These changes are not completed until 45 to 60 days after calving. In spite of the recommendation to wait 60 days after calving to breed cows, it was found that 35 per cent of the 85 farmers were breeding their cows less than 50 days after calving. Although the fertility results have not been completely tabulated on these herds, they were, no doubt, similar to those shown in Table 1 for the LSU herd.

TABLE 1.—The relationship between time bred after calving and services per conception in the LSU Holstein herd (1931-1946)

	Time bred after calving (days)				
	1-40	41-60	61-90	91 or more	Total
No. of services	78	153	311	459	1,001
No. of calves	33	85	180	267	565
No. of services per calf	2.36	1.80	1.73	1.72	1.77

It will be noted in Table 1 that more services were required per calf when cows were bred less than 60 days after calving, particularly when cows were bred within 40 days after calving.

The question often arises as to whether a dairyman is justified in breeding his cows less than 60 days after calving in an effort to have them calve at some desired time. The time of freshening or calving is very important for most Louisiana dairymen, since their base milk pricing period is from September 1 to January 1. Granting that they sacrifice some in fertility by breeding cows less than 60 days after calving, as shown in Table 1, the question then is whether or not they gain any time following such a practice. This question is answered by the results adapted from Trimberger's report (27).

TABLE 2.—Conception rate at various intervals after calving and days from calving to conception

Interval from calving to first service	Per cent conception	Days from calving to conception
50 days or less	31	100
51-60 days	67	75
61-90 days	70	94
More than 90 days	76	130

These results definitely show that when cows are bred within 50 days or less following calving, more days are required for conception than when cows are bred 51 to 90 days after calving. In other words, time is lost, not gained, by breeding cows too early. The dairyman

should wait at least 50 days after calving, preferably 60 to 90 days, to breed his cows in order to obtain the highest fertility and save the most time.

Timing of Service During the Heat Period—It has been known for several years that the highest fertility is obtained when cows are bred from the middle to the end of the heat period. In the temperate regions of the world, it has been found that the heat period usually ranges from 2½ to 28 hours. In fact, it has been reported that 93 per cent of the cows will fall within the range of 13 to 27 hours. However, results on 129 cows and heifers of the Holstein and Jersey breeds in the LSU herd show an average of 12 hours for the duration of heat, with a range of 3 to 36 hours. These results indicate that the average duration of heat in Louisiana, which is a subtropical area, is from 5 to 6 hours shorter than that reported in the temperate regions.

Table 3 gives a practical guide for inseminators. This guide takes into consideration the relationship between the time of service and the time of ovulation, or release of the ripe egg, and the length of time the spermatozoa and egg will remain fertile in the cow's reproductive tract.

In order to put the information in Table 3 to its greatest use, the dairyman or beef cattle producer must check cows for heat at least twice daily, preferably three times a day, and report to the breeding technician the exact time the cow was first observed in heat. Three times a day is recommended, not only to obtain the highest fertility but also because studies in Louisiana show that many cows are missed by twice daily checks. This results in unnecessary delays in getting many cows in calf and the loss of money if the cow does not freshen at the proper time. Another point which should be emphasized is that some cows have "silent" heats or show very few signs of heat. Such cows require very careful observation by the herdsman. The herdsman will find that a heat expectancy chart is very useful in making these thrice daily observations for heat. Most cows have estrus cycles of 16 to 24 days, averaging about 21 days. Thus, after a cow has had two or more heat periods, one can predict quite accurately when she will return to heat.

TABLE 3.— Time to breed cattle artificially for best results

Heat first observed	Best time to breed	Too late for good results
Morning (before 9 a.m.)	The same day (afternoon)	Next day
Forenoon (9 a.m. to 12 noon)	Late the same day or early next day	After 10 a.m. next day
Afternoon and early evening (night milking)	Next morning (forenoon)	After 2 p.m. next day

TABLE 4.—The relationship between age of cow and services per calf in the LSU Holstein herd (1931-1946)

Number of calf	Number of cows	Number of services per calf
First calf (heifers)	370	1.61
Second calf	249	1.76
Third calf	186	1.82
Fourth calf	131	1.73

Age of Cow—Many workers have presented evidence that the fertility of dairy cows varies with age. Also, it appears that many Louisiana dairymen and breeding technicians are of the opinion that heifers are more difficult to settle in calf than are mature cows. Table 4 presents results on the relationship between age and the number of services required per calf in the LSU Holstein herd. Statistical analysis of these data revealed no significance among these different age groups. Therefore, it appears that virgin heifers will conceive just as readily as older cows.

Seasonal Effects—From the outset of the artificial breeding program in Louisiana, it has been observed that the quality and fertility of semen of bulls are affected by the high temperatures and humidities during the summer months. The proportion of abnormal

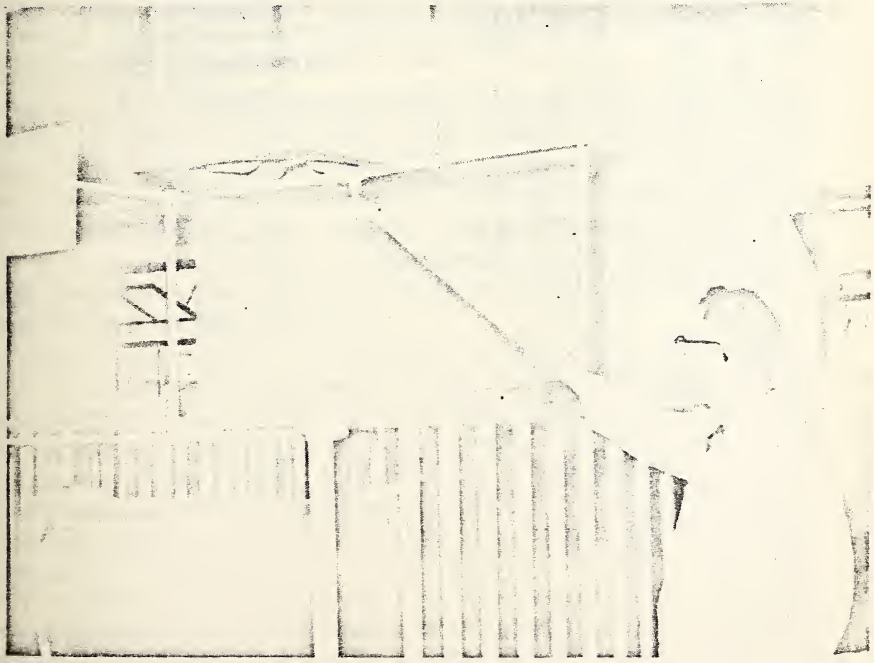

FIG. 16.—View of LSU barn showing fan and sprinkler used for cooling bulls during summer months.

spermatozoa increases and initial motility of the spermatozoa decreases in the summer and early fall months, resulting in many ejaculates being unfit for artificial insemination purposes. Studies (24) have been conducted for the past five years on artificial cooling of bulls in an effort to improve this situation. Some beneficial results from cooling have been obtained, but these investigations are still in progress and no definite statements can be made.

That the fertility of cattle varies with seasons of the year in Louisiana has been observed for some time now. In artificial breeding (19), the fertility has been the lowest during the summer and fall and the highest during the spring. Table 5 gives the results in the LSU herd.

TABLE 5.—Seasonal variation in fertility in the LSU Holstein herd (1931-1946)

Seasons	Number of first services	Per cent 60-90 day non-returns
Spring (March, April, May)	428	72.7
Winter (December, January, February)	712	69.9
Fall (September, October, November)	421	66.0
Summer (June, July, August)	105	51.4

Natural service was practiced during this time. Although results in artificial breeding have not been as marked as those shown in Table 5, they have shown a similar trend. Therefore, it definitely appears that climate has some effect on the fertility of cattle. This effect is now under investigation.

Diseases—It is generally believed that any disease which affects the health of a cow will also affect her fertility. However, certain diseases have rather specific effects on reproductive efficiency. Among these diseases are brucellosis, or Bang's disease, vibriosis, trichomoniasis, and granular vaginitis. These diseases are often referred to as genital or venereal diseases of cattle. The organisms causing the first three of these diseases are harbored in the uterus and can affect fertility materially.

During recent years, the incidence of brucellosis has ranged from 6 to 14 per cent of all cattle tested by the state and federal veterinarians in Louisiana. However, it was found in only 2 per cent of the "problem" cows in the 85 herds mentioned previously. Many dairymen are of the opinion that abortion is the primary effect of Bang's disease. It must be emphasized, however, that this disease is not the only cause of abortion in cattle, nor do all cows having this disease abort. On the average, Bang's positive cows are more difficult to settle in calf. Information on brucellosis control and eradication programs is available from county agents and veterinarians.

Vibriosis, or *Vibrio fetus,* is a genital disease which causes re-

peat breeding, delayed breeding, sterility, and abortion. Abortions are usually earlier than those in Bang's disease animals. As is the case with Bang's disease, not all infected cows abort. Since testing programs are not highly accurate, the incidence of vibriosis in Louisiana is unknown. However, the symptoms of repeat breeding and abortion in many herds indicate that this disease is a factor in the state. Five per cent of the "problem" cows tested by the cervicovaginal mucus agglutination test in 85 herds gave a positive reaction. Also, the *Vibrio fetus* organism has been isolated from aborted calves. This is proof that the disease is in Louisiana cattle, but it does not give any conclusive evidence as to its prevalence. When this disease is suspected in a herd, the farmer should consult his veterinarian immediately, since it can mean the difference between a very poor calf crop and a satisfactory level of fertility.

Only a very limited number of cases of trichomoniasis have been diagnosed in Louisiana. In fact, only two cases were found in the 85 herds studied from 1953 to 1956. However, dairymen and veterinarians should be on the lookout for this disease, since it can cause low fertility and abortions.

Granular vaginitis, which is a venereal disease that primarily affects the vulva and vagina of the cow, is quite prevalent in Louisiana. It was found in 26.4 per cent of the 383 "problem" cows examined in the 85 herds. It should be emphasized, however, that it was just as prevalent in pregnant as in non-pregnant cows. This raises some questions as to the effect of granular vaginitis on fertility. Most authorities agree now that, unless the infection is severe, it has very little or no effect on fertility.

Several diseases, such as leptospirosis and anaplasmosis, affect the general health of an animal and can affect fertility also. In fact, the high fever which often accompanies leptospirosis, in particular, has been observed to cause many abortions, retained afterbirths, and repeat breedings in some of the herds studied. The incidence of leptospirosis was found to be 7 per cent in the herds studied.

Physiological Disorders—The most common physiological disorders affecting fertility observed in Louisiana cattle have been anestrus and cystic ovarian follicles. In the 85 herds studied, 8.2 per cent of the "problem" cows were classified as being anestrus. Anestrus cows were those that had not been in heat for an extended period after calving, heifers that showed no heats, and bred cows that were found to be open when examined for pregnancy. Two primary causes of anestrus were found to be persistent corpus luteum, or "yellow body," and inactive ovaries. Cystic ovarian follicles were found in 5.3 per cent of the "problem" cows. Most of these cows came into heat at very frequent intervals, less than 15-day cycles, while some were "chronic bullers." The chronic bullers remain in

heat almost continuously. Fertility in such instances is very low. Each of these difficulties requires veterinary attention.

Feeding and Management—Feeding practices can and do affect fertility in some cases. However, it should be emphasized that when the feeding was anywhere near adequate, no relationship was found between feeding practices and fertility in the herds studied. Under these feeding conditions, adequate quantities of vitamin A or provitamin A (carotene) and phosphorus were being fed. Feeding supplementary vitamin E in the form of wheat germ oil and similar products has shown no beneficial effects on fertility. When cattle are fed a balanced grain ration with plenty of green forages in the form of pasture, hay, silage, and soilage, along with the necessary minerals either in the grain mixture or as a supplement, it would appear that adequate nutrients for high fertility are obtained.

Several management practices that are related to the fertility of cattle have already been mentioned in connection with time to breed cows and diseases. In addition to these, breeding methods, replacements, and herd sanitation should be discussed briefly. Studies in Louisiana show that 41.3 per cent of the dairymen using artificial breeding use a combination of bulls in natural service and artificial insemination. If there are any genital diseases present in these herds, this is an excellent method of spreading them. It has been established definitely that a bull can spread brucellosis, vibriosis, trichomoniasis, and granular vaginitis through the physical contacts he makes in natural mating or through his infected semen. On the other hand, when artificial insemination is done properly, the bull is removed as a spreader of these diseases.

The Louisiana studies also show that 70 per cent of the dairymen buy replacements at least every two years. Thirty per cent of those buying replacements do not have the cattle tested for diseases that affect fertility. This practice can lead to many breeding difficulties.

The primary factor in herd sanitation is care of the cow at and immediately after calving. One should make sure that the cow is properly cared for and that she does not retain the afterbirth. Abnormal discharges of blood and pus which persist after calving should be observed and called to the attention of the veterinarian and breeding technician.

The more important management faults found in Louisiana agree with those reported by Asdell for New York (4). They are summarized as follows:

1. Failure to detect signs of heat and to breed at the most favorable time.
2. Breeding back too soon after a cow has calved. Sixty days is soon enough if the cow has cleaned properly. Earlier breeding has a lower percentage of success.

3. Failure to call in the veterinarian at the first sign of trouble. These signs of trouble are as follows: (a) absence of heat periods, (b) presence of any unnatural discharges, (c) heat periods of fewer than 15 days or continuous heat, (d) heat periods irregular or at intervals of more than 28 days, (e) cows or heifers receiving three or more services without conceiving, (f) abortions at any time, and (g) retained placenta.
4. Changing bulls when a cow does not conceive to a first or second service or using both natural and artificial service.
5. Purchasing replacements without health records.

Although little is known about many of the factors which affect fertility of cattle, fertility can be improved considerably by putting into practice what is already known. This requires proper feeding and management by the dairyman, cooperation between the breeding technician and dairyman, good breeding technicians, veterinary services when needed, patience, and highly fertile bulls in the stud.

Selecting Sires for Use in Artificial Insemination

The primary objective of artificial insemination is herd improvement. Good bulls are where you find them, and the good ones will transmit high production and desirable type when placed in artificial service regardless of the line of breeding or name. "A rose by any other name would smell the same." Genetic ability is the important factor. Everyone likes a bull that looks good, but it must be remembered that there is practically no correlation between type of a bull and production of his daughters. Likewise, there is very little correlation between the type of a bull and the type of his daughters. It is much more important to find out how his sisters and daughters look and produce. A "Fancy Pedigree" is often overemphasized. Pedigrees are very important but may also be misleading. Variation in environmental conditions on farms makes pedigree evaluation quite difficult. Production alone is not the answer. The dairyman wants large straight cows with good feet and legs and well-attached udders. He is not interested in a cow that milks well for one or two lactations, then has to be culled because the udder goes to pieces.

Proved Sires

There are many definitions of a proved sire. However, there are two different types of proof. "Natural" proof is based on 10 or more unselected daughter-dam comparisons. The daughters are sired by natural mating and are usually located in one or two herds. "A B" proof is based on 40 or more unselected artificially sired daughters which are located in many different herds. "A B" proof is usually lower than "natural" proof since the records are made under average farm conditions which may include all levels of feeding and

management. However, "A B" proof is a better estimate of a bull's transmitting ability.

Proved sires vary a great deal in their genetic ability. The integrity of the owner must not be overlooked. It is important to determine what portion of the daughter's production is due to environment and how much is due to the genetic ability of the bull. One must also find out whether all tested daughters are included in the proof, or if it is based on selected records. The best "natural" proof is where the daughters and their dams are in the same herd and have had the same treatment. Most authorities agree that the level of production is determined largely by feed and management, with breeding accounting for only about 25 per cent of the production. Thus, a bull whose daughters average 8,000 pounds of milk and 400 pounds of fat under ordinary farm conditions may be equal or superior to a bull whose daughters have a much higher level of production under a more nearly ideal environment.

Research workers in New York compared "natural" proof with "A B" proof on 50 Holstein sires. The sires were ranked in four groups according to the level of their "natural" proof. The results are shown in Table 6.

TABLE 6.—Comparison of "natural" proof with "A B" proof

Ranking of sires	Average of natural daughters	Average of AB daughters
	(lbs. fat)	(lbs. fat)
Top 25%	499	435
2nd 25%	472	440
3rd 25%	460	419
4th 25%	450	419
Over-all average	470	428

This table emphasizes the effects of different kinds of environment. "A B" daughters are usually in commercial herds with all types of conditions, while "natural" proofs are more often made in superior environments.

Selected Young Sires

Do sires with "natural" proof have better "A B" daughters than "selected" young bulls? With this question in mind, Pennsylvania workers made a comparison between the two groups and found essentially no difference in production or type. The results are shown in Table 7. This study indicates that "carefully selected" young bulls are just as reliable as bulls with "natural" proof.

Some of the points to be considered in selecting young bulls are:

1. The sire and grandsires should be proved for desirable type and high production. ("A B" proof is highly desirable.)
2. The dam and granddam should have several good records (un-

TABLE 7.—Comparison of "A B" daughters of "natural" proved sires with "A B" daughters of carefully selected young bulls

Breed	No. of bulls	Natural daughters fat. av.	diff.	"A B" daughters fat av.
		PROVED SIRES		
Holstein	22	474	+56	421
Guernsey	7	470	+35	367
Jersey	6	390	+52	422
		YOUNG SIRES		
Holstein	13			422
Guernsey	18			377
Jersey	15			413

selected). It is also desirable for them to have three or more tested sons or daughters reasonably good in type.

3. Much can be learned about a young bull by studying his half-sisters and half-brothers.
4. Size is an inherited trait and is thought to be a big factor in relation to production.
5. The young bull should have reasonably good body conformation.
6. Last but not least, the herd from which the sire is selected should be noted for continuous high production and good type. The pedigree of a "carefully selected" young bull is shown in Figure 17. His sire and grandsires are examples of desirably proved bulls.

Health of Bulls

Bulls are tested for all known reproductive diseases, including brucellosis, tuberculosis, vibriosis, trichomoniasis, and leptospirosis before they are placed in service and at regular intervals thereafter. As an added precaution, the health of the herd from which the bulls are purchased is investigated. Tests are also made to determine semen quality and potential fertility of each bull.

Records and Reports Used in Artificial Insemination

Adequate records and reports are essential for the successful development of an artificial insemination program. Records kept by artificial insemination organizations may be used for many purposes. First and foremost, complete and accurate financial and breeding records are necesary for the routine operation of the association. It would be impossible to operate efficiently without up-to-date breeding efficiency records for technicians and bulls. These reports enable the management to curtail or discontinue service from bulls that do not have a satisfactory level of fertility. The relative efficiency of each technician is also indicated by these reports. This gives the field supervisors an opportunity to spend more time

B D I PABEMPGOV ALGY—1281300
Born: 1-30-56
Bred by: Dairy Research Branch,
U. S. D. A.,
Beltsville, Maryland
All records 2 x 305 M.E.

PABST RAMBLER WALKER—975138
—"VG"
Silver Medal Production & AI
Proved Sire.
1,359 AI Daus. av. 13,340M 3.6% 478F
61 Daus. av. 15,063M 3.7% 553F
61 Dams av. 14,125M 3.7% 526F
+938M +27F
114 Cl. Daus. av. 80.0% 100.7 BAA%

PABST RAMBLER 916876
4 daus. av. 18,545M 3.5% 646F
78 cl. offspring: 4E, 24 VG, 38 GP, 13 G, 1F. SON OF: Pabst Roamer "EX" Gold Medal Sire.

PESTER INEZ DEAN ORMSBY—2054580
2 rec. av. 13,590M 3.6% 493F

B D I EMPGOLIN ANNETTE—3507143
3 rec. av. 19,787M 3.7% 741F
Best rec. 22,236M 3.7% 812F
19,336M 3.7% 706F 3-3 305 2x (actual)
National record for milk in this class.

S J C VALLEY EMPEROR STAR—857269—"VG"
Silver Medal Production & AI Proved Sire
52 daus. 13,614M 3.8% 519F
52 dams 12,828M 3.7% 478F
+786M +.1% +41F
862 AB daus. av. 11,486M 3.8% 433F

B D I GENLINO ANNETTE—2817080
2 rec. av. 18,048M 3.9% 711F
Best rec. 18,987M 3.9% 733F

FIG. 17.—Pedigree of an outstanding young sire.

in the areas where their assistance is needed most. Monthly reports that include the number of cows inseminated and the breeding efficiency for each local unit are published. This gives the technicians and officials of the locals an opportunity to compare their results with those of other areas.

Secondly, the Purebred Dairy Cattle Association has established certain requirements governing artificial insemination of purebred dairy cattle. Some of the more important requirements are as follows: (1) The bull stud must sign a contract agreeing to carry out all rules and regulations of the PDCA, and the manager and all technicians must be certified. (2) All bulls must be blood typed before being placed in service. (3) Permanent records of semen collections and shipments must be kept. (4) All technicians must use the PDCA approved breeding receipts. (5) Complete individual herd breeding records, including carbon copies of breeding receipts, must be retained for a period of at least three years. (6) Tubes of semen must be accurately labeled at all times. (For more detailed information including "Instructions to the Inseminators," see the official PDCA regulations.)

Thirdly, artificial insemination records and reports furnish valuable information to agricultural extension agents, research workers, teachers, and writers. Records kept by the local officers and technicians include the following: (1) treasurer's monthly financial report, (2) inseminator's daily record and cash receipts, (3) indi-

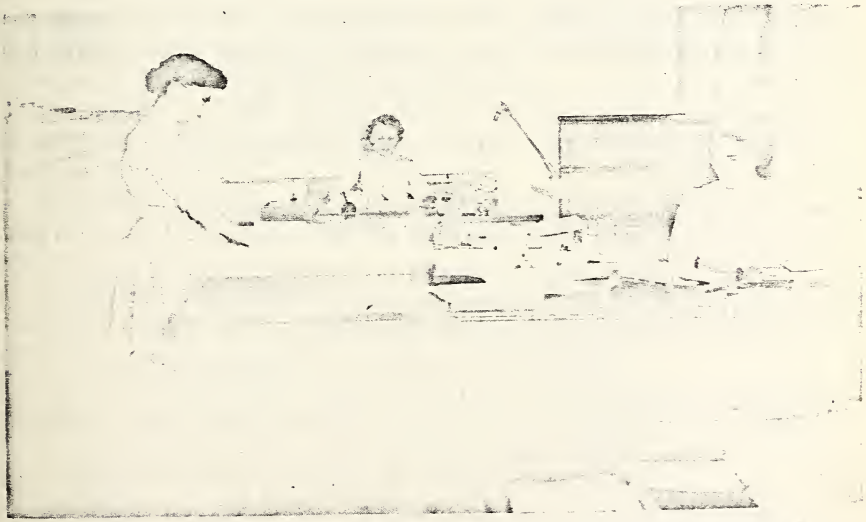

FIG. 18.—A view of the record room at the Dairy Improvement Center. The name of the cow, date of insemination, bull used, and other information is transferred from the breeding receipts to machine cards for analysis. Complete and accurate information is readily available for research, routine operation, and monthly and annual reports.

vidual farmer's ledger, (4) individual farmer's herd record, (5) barn breeding records, (6) breeding receipts, and (7) membership agreements. Copies of the breeding receipts, semi-monthly summary sheets, copies of the agreements for new members, and treasurer's reports are forwarded to the central office at regularly designated intervals.

Complete and accurate records are necessary for the efficient operation of an artificial insemination organization. The technician is a very important "spoke in the wheel" of a record-keeping system. He must be honest, thorough and efficient in his techniques of inseminating as well as in his record keeping. There is no place for shortcuts or putting off until tomorrow what should be done today in either of these important phases of his work.

Summary of Important Facts Relating to Artificial Insemination

1. The first artificial breeding association in the United States was organized in 1938 at Clinton, New Jersey, with 102 members and 1,050 cows enrolled.

2. By January 1, 1960, artificial insemination was being used in every state in the Union, with a total of 6,932,294 cows being inseminated in 946,000 herds during 1959.

3. Artificial insemination of dairy cattle was started on a statewide basis in Louisiana on October 15, 1947, with the program being administered jointly by the Louisiana Artificial Breeding Cooperative, Inc., and the LSU College of Agriculture.

4. By January 1, 1960, there were 42 local circuits employing 45 breeding technicians and more than 51,000 cows being inseminated annually in the state.

5. In artificial insemination, the usefulness of outstanding sires may be increased manifold. On the average, bulls used in organized breeding associations are bred to more than 2,800 cows annually.

6. A general knowledge of the structure and functions of the reproductive organs of the male and female is essential in order to master the techniques used in artificial insemination.

7. Use of the artificial vagina has been found to be the most practical and satisfactory method of collecting semen from the bull.

8. Good quality semen from highly fertile bulls is one of the primary requirements for success in artificial insemination.

9. No single test has been devised that will accurately measure semen quality and reliably predict its potential fertility. However, the following combination of tests and minimum standards are recommended for semen evaluation: (1) initial progressive motility of at least 50 per cent, (2) a concentration of at least 500,000,000 sperm per ml., and (3) a modified methylene blue reduction time of

nine minutes or less. Previous breeding records are also a valuable aid in predicting the fertility of a bull.

10. Any delay in extending semen and starting the cooling process will cause a decrease in quality and an increase in bacterial growth.

11. For best results, liquid semen should be cooled gradually and stored at 40° F.

12. Egg yolk-citrate and heated milk are the most widely used fluids for extending semen.

13. When penicillin and streptomycin are added to extended semen, they not only increase fertility but control bacterial growth, improve livability of the spermatozoa, and act as metabolic depressors.

14. High quality semen can be extended at a ratio of 1:100 or to contain 7,000,000,000 live sperm per ml. without any decline in fertility.

15. The desired temperature of 38° to 40° can be maintained in shipment provided the semen is properly packaged.

16. Properly prepared semen can be frozen and stored at —79° C. (—110° F.) or colder for an indefinite period with little or no decline in fertility.

17. The deep cervical or intra-uterine method of insemination has been found to be the most satisfactory technique.

18. One ml. of extended semen deposited in the middle of the cervix has been found to give optimum results. When the insemination tube is passed halfway through the cervix, there is less chance of infection, and in case the cow is with calf, pregnancy is not likely to be interrupted.

19. Pregnancy in cattle can be accurately diagnosed as early as 35 days by an experienced person.

20. Fertility was found to be only 10.6 per cent heritable, thus indicating that most breeding difficulties are environmental in nature.

21. The cow should have a sexual rest of at least 50 days after calving. Time is actually lost, not gained, by breeding cows too soon.

22. Cows should be checked for heat at least twice and preferably three times a day.

23. For best results, cows should be inseminated between the middle and the end of the heat period.

24. Age of the animal is not an important factor affecting fertility. When the non-breeders are disregarded, heifers conceive just as readily as older cows.

25. Season is an important factor affecting fertility of cattle. The level of fertility in Louisiana is highest in the spring and lowest in the summer, with fall and winter being intermediate. Bulls show a marked decline in semen quality during the late summer and early

fall. Some beneficial results have been observed when bulls were cooled by artificial means.

26. The most important genital diseases affecting the fertility of Louisiana cattle are brucellosis, vibriosis, trichomoniasis, and vaginitis.

27. The two most common physiological disorders affecting fertility were found to be anestrus and cystic ovarian follicles.

28. Feeding practices do affect fertility in isolated cases. However, where nutrition was anywhere near adequate for growth and production, it was not a factor in reproduction.

29. Artificially sired daughters of "carefully selected" young bulls are equal in production and in type to artificially sired daughters of sires selected on the basis of "natural" proof.

30. "A B" proof, based on artificially sired daughters located in many herds, is the most reliable estimate of a bull's transmitting ability.

31. Complete and accurate records are necessary for the successful operation of an artificial insemination program.

32. Much of the success achieved in artificial insemination can be attributed to research. Likewise, the future of this program is dependent upon the development of new and improved techniques.

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